Reaction Kinetics and Pathway of Hydrothermal Decomposition of Aspartic Acid

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ABSTRACT: The kinetics and pathway of hydrothermal decomposition of aspartic acid were studied using a continuous-flow tubular reactor. The reaction was carried out in the temperature range of 200–260°C and at a pressure of 20 MPa. Deamination was the primary reaction, indicated by the presence of significant amount of ammonia, fumaric acid, or maleic acid in the products. Other reaction products were pyruvic acid, malic acid, and traces of succinic and lactic acid. Traces of alanine were also detected, showing the possibility of decomposing high-molecular weight amino acids to obtain simple amino acids such as glycine or alanine. Results on the effect of reaction parameters demonstrated that decomposition of aspartic acid is highly temperature dependent under hydrothermal conditions. For a slight temperature difference of 60°C (from 200 to 260°C), the first-order reaction rate constants of 0.003 significantly increased to 0.231 s^{-1}. The activation energy was 144 kJ/mol, as calculated by the Arrhenius equation. No significant effect was exhibited by other reaction parameters such as pH and pressure. The results are useful in controlling the hydrolysis of proteinaceous materials toward high yield of aspartic acid under hydrothermal conditions. © 2007 Wiley Periodicals, Inc. Int J Chem Kinet 39: 175–180, 2007

INTRODUCTION

The potential use of water as a medium for various organic chemical reactions is being explored because of its fascinating properties at high-temperature and high-pressure conditions. It also offers ecological benefits if used as a solvent in industrial processes. Reactions in sub- and supercritical water have attracted considerable interests in recent years [1–3]. Numerous works

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on the applications of the sub- and supercritical water to chemical synthesis and decomposition have been reported [4]. Related research topics include the production of cellulose and its derivatives from agricultural wastes [5], recovery of useful monomers such as ethylene glycol and terephthalic acid from polyethylene terepthalate (PET), and polyvinyl alcohol (PVA) [6]. The water at subcritical conditions has also been successfully applied to recover L-lactic acid from poly(L-lactic acid) [7]. In food-processing technologies, the superheated water as an environmentally benign and safe solvent has been used to extract isoflavones from soybean [8].

Most of our recent works on the application of high-temperature and high-pressure water deal with the recovery of amino acids from proteinaceous wastes [9,10], combining the benefits of recycling and waste treatments. Amino acids have wide uses and applications in pharmaceuticals, food products, animal nutrition, and cosmetic industries. They can be used to treat various diseases such as those of the renal, gastrointestinal, endocrinal, and dermal systems among others. In food industry, amino acids are utilized as taste enhancers and animal feeds. If each amino acid could be separated individually, these could also be used as reagents for the synthesis of new materials including electronic-related chemicals (e.g., for liquid crystals, exposure liquids for color copiers).

However, the yield of amino acids using only water without any additives was still low compared to the quantity of the amino acids originally present in the raw materials [10–13]. It was likely that the produced amino acids decomposed quickly in high-temperature and high-pressure water. In addition, the hydrolysis of protein was not completed to produce amino acids. Thus, information on the decomposition of amino acids is necessary in optimizing the conditions for the hydrolysis of proteinaceous materials toward high yield of the desired amino acids. One amino acid of interest to us is aspartic acid (Asp), which is one of the easily degradable amino acids. To date, the reaction kinetics and decomposition of some amino acids have been reported in our previous work [14]; however, a detailed discussion on Asp decomposition has not been conducted yet.

Asp is a hydrophilic amino acid that can be found in high levels throughout the main body and helps protect the liver from some drug toxicity and the body from radiation. The most recent and popular application of Asp is as the sweetener called aspartame, which is a combination of Asp and phenylalanine [15]. Asp can also be used as a material for the production of biodegradable plastics [16]. Fox [17] has reported that Asp is the key amino acid for thermal copolymerization. Furthermore, Asp can be considered as a model in the study of stability of hydrophilic amino acids at elevated temperatures.

The primary objective of this work is to determine the kinetics of hydrothermal decomposition of Asp and to elucidate decomposition pathway based on the reaction products.

MATERIALS AND METHODS

Materials

In each experiment, L-aspartic acid (Nacalai Tesque) was diluted to 30 mM using Milli-Q water. Prior to each experimental run, the sample solution and Milli-Q water were degassed for about 40 min by ultrasonic wave. All other reagents were supplied by Hayashi Kasei Co. (Shizuoka, Japan).

Experimental Apparatus and Procedures

All experiments on decomposition of Asp were carried out over the temperature range of 200–260°C at a reaction time of 15–360 s. Unless otherwise specified, the pressure was held constant at 20 MPa, enough to maintain the reaction mixture in the liquid phase. In each experiment, helium gas was introduced into the sample containers to avoid air to be dragged along the reactor.

All experiments were conducted in a high-pressure, isothermal plug-flow tubular reactor capable of continuous operation at temperatures up to 450°C and pressures up to 30 MPa. The experimental apparatus is shown schematically in Fig. 1. Water and the sample were delivered by an HPLC pump (JASCO, PU-1580). To minimize the effect of the transition in temperatures, the water was preheated (OD: 1/16 inch, ID: 0.5 mm, length: 20 m, volume: 3.93 cm^3) at the reaction temperature. The hot water was mixed with the sample at a volumetric ratio of 1:2, resulting in a 10-mM Asp

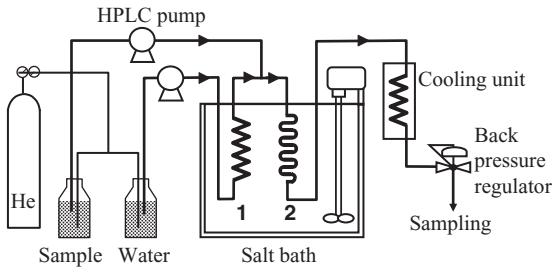

Figure 1 Schematic diagram of continuous-flow tubular reactor apparatus for experiments with high-temperature and high-pressure water (1: preheater and 2: reactor).

solution prior to entering the main reactor (OD: 1/16 inch, ID: 0.25 mm, length: 20 m, volume: 0.98 cm^3). At the exit of the reactor, the reaction effluent was quenched in a water bath (ADVANTEC, model LC-101) at a temperature of 5°C. The pressure was reduced to atmospheric conditions by a back-pressure regulator (JASCO, SCF-Bpg). A sample of about 30–50 cm^3 was collected after reaching steady state, usually after about 30 min.

The reaction time in this study is the residence time of the mixture inside the reactor and was calculated by considering the flow rates of water and Asp solution. On the basis of material balance at the input and output of the reactor, an equation for the residence time can be obtained in a simple form.

$$\tau = (\rho'_w/\rho_w)V_R/(Q_w + Q_{Asp}) \quad (1)$$

The residence time (τ) is in s, the volume of the reactor (V_R) is in cm^3, the volumetric flow rates of water (Q_w) and diluted solution of Asp (Q_{Asp}) are in cm^3/s, and the density of water at ambient (ρ_w) and reaction conditions ($\rho_{w'}$) is in g/cm^3. Since diluted sample solution of Asp was used in the experiment, it was assumed that the density of the Asp sample solution is the same as that of water.

Analysis of Amino and Organic Acids

Amino and organic acids contents of reaction effluents were determined using amino acid analyzer (LC-10AD, Shimadzu Corp.) and organic acid analyzer (LC-10A, Shimadzu Corp.), respectively. Amino acid analyzer is a combination of an ion-exchange column (Shim-pack Amino-Na, Shimadzu Corp.) and postcolumn labeling methods with spectrophotometer (RF-10A, Shimadzu Corp.). In sample preparations for amino acid analysis, filtration was done using ultrafiltration membrane (30,000 fractional molecular weight, Millipore Ultra Free C3) to maintain the performance of the analytical system. The quantities of 17 amino acids—namely, aspartic acid (Asp), threonine (Thr), serine (Ser), glutamine (Glu), proline (Pro), glycine (Gly), alanine (Ala), cystine (Cys), valine (Val), methionine (Met), isoleucine (ILeu), leucine (Leu), tyrosine (Tyr), phenylalanine (Phe), histidine (His), lysine (Lys), and arginine (Arg)—could be determined in each sampling run. The organic acid analysis system consists of an ion-exclusion column (Shim-Pack SCR-102H) and electroconductivity detector (CDD-6A). The objects to be analyzed are aliphatic carboxylic acids, hydroxycarboxylic acids, ketocarboxylic acids, and other organic acids having dissociation constant (pKa) of 2–5 and carbon number of 5 or less.

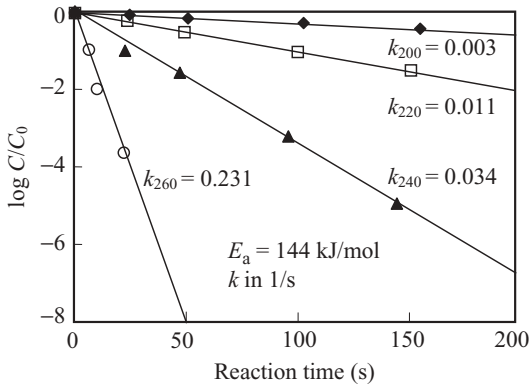

Figure 2 First-order kinetic plots of hydrothermal decomposition of aspartic acid (♦: 200°C, □: 200°C, ▲: 240°C, and ○: 260°C).

RESULTS AND DISCUSSION

Reaction Kinetics

The first-order kinetic plots over the temperature range of 200–260°C are shown in Fig. 2. Results show that the temperature has a significant effect on the decomposition of Asp. An increase in reaction temperature from 200 to 260°C dramatically increased the first-order reaction rate constant from 0.003 to 0.231 s^{-1}. The activation energy in the temperature range of 200–260°C has been estimated at about 144 kJ/mol by using the Arrhenius equation. This activation energy is lower than that for the decomposition of glycine (166 kJ/mol), alanine (154 kJ/mol), and serine (149 kJ/mol) [14]. It can be suggested that Asp is the one of the easiest decomposed amino acids.

Figure 3 shows the concentration profile of the Asp decomposition products against reaction time at

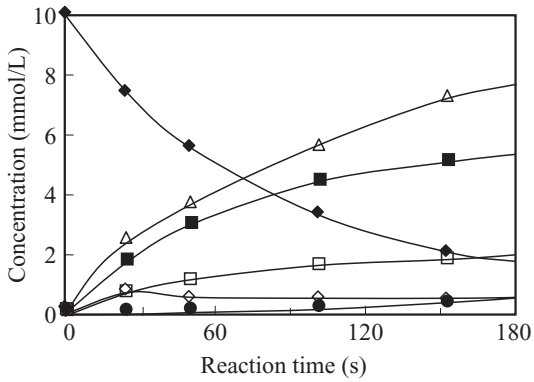

Figure 3 Effect of reaction time on the concentration of products obtained by hydrothermal decomposition of aspartic acid at 220°C (♦: aspartic acid, △: ammonia, ■: fumaric acid, □: maleic acid, ◇: pyruvic acid, and ●: malic acid).

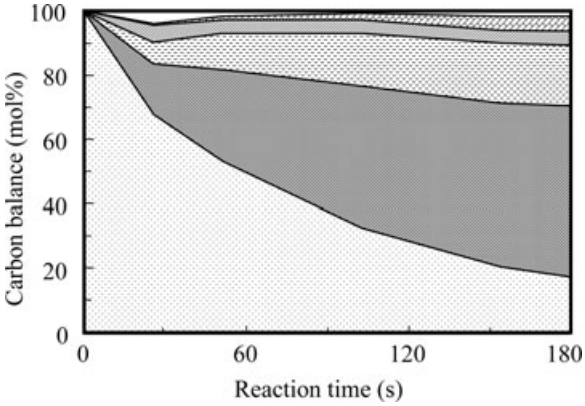

Figure 4 Total organic carbon distribution in the products obtained at 220°C (▭: aspartic acid, ■: fumaric acid, ▨: maleic acid, ▬: pyruvic acid, ▨: malic acid, and ▭: others).

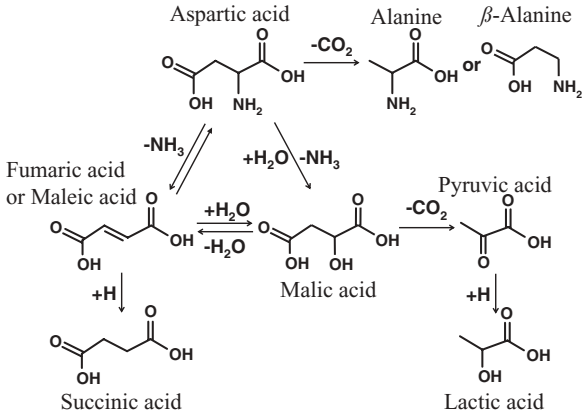

Figure 5 Degradation pathway of aspartic acid under hydrothermal conditions.

reaction temperature of 220°C. Significant amount of ammonia was produced, indicating that deamination was the predominant reaction. The above results agree with that of Bada [18,19], who first reported that the major decomposition pathway for Asp in aqueous solution is deamination to produce fumaric acid and ammonia. Other than ammonia, low-molecular weight carboxylic acids such as fumaric acid, maleic acid, pyruvic acid, and malic acid were also present in considerable amounts. The carbon balance and distribution in the products were checked, and the results are presented in Fig. 4. "Others" in the figure indicates the carbon in trace amounts of succinic acid, lactic acid, and Ala. It has been reported that most amino acids having simpler structure and higher decomposition temperature are more stable under hydrothermal conditions [20]. However, our results showed that Asp decomposed very fast with increasing temperatures. This suggests that hydrolysis enhances the decomposition of Asp. Sato [20] also reported that the decomposition rates of hydrophilic amino acids tend to be higher than those of hydrophobic ones, and thus the thermal stability of amino acid is not dependent on its molecular weight. In addition, the decomposition rate of Asp is expected to be higher at the hydrogen fugacities of the redox buffered experiment [21].

Reaction Pathway

On the basis of the experimental results in Fig. 4, the main reaction pathway of the hydrothermal decomposition of Asp is shown in Fig. 5. Deamination to form fumaric or maleic acid is the predominant reaction as demonstrated by the high concentration of these products in Fig. 4. The deamination of Asp to fumaric acid is a reversible reaction. Deamination of Asp differs from decomposition of most amino acids because it could take place enzymatically and nonenzymatically [22]. Of the 20 amino acids found in protein, only aspartic acid and asparagine are known to deaminate even in the absence of enzymes [19].

Malic acid was a product of hydrolytic deamination of Asp or hydrolysis of fumaric (maleic) acid, the latter being a reversible reaction. Pyruvic acid may have been formed from decarboxylation of malic acid. Further hydration of pyruvic acid would result into formation of lactic acid, which was present in trace amount. A separate experiment performed for the decomposition of malic acid at 240°C (20 MPa) confirmed the formation of pyruvic acid and lactic acid. Maleic acid, which is the product of hydrogen abstraction from malic acid to form a double bond, was also found in the product. The presence of trace amount of succinic acid was most likely due to hydration of fumaric or maleic acid.

Traces of Ala or β-Ala formed by decarboxylation of Asp were also detected. This result indicates a possibility of obtaining simple amino acids such as Gly and Ala from decomposition of relatively high-molecular weight amino acids such as Asp. This interesting phenomenon merits further investigation.

Effect of pH and Pressure on Decomposition Rate

Acidic condition enhances the rate of decomposition of Asp as shown in Fig. 6. The rate constant of 0.022 at pH 9 increased to 0.034 at pH 2.7. Bada and Miller [19] have showed that the deamination of Asp in buffered aqueous solution at 60–135°C is pH independent for

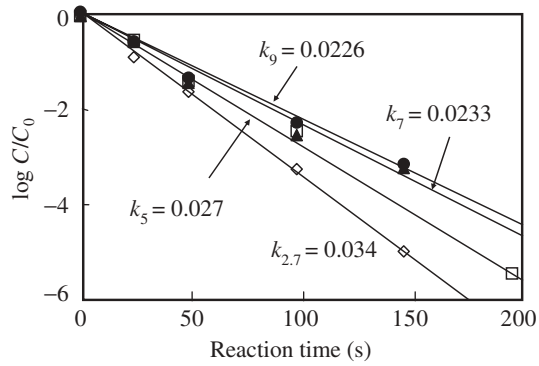

Figure 6 Effect of pH on decomposition rate of aspartic acid at 240°C and 20 MPa (◇: pH 2.7, □: pH 5, ▲: pH 7, and ●: pH 9).

pH range of 4.5–7 and for pH values greater than 10. Previous studies also showed that the pH of reaction media has an effect on the hydrolysis and cyclization of dipeptide [23] and reaction selectivity of deamination and decarboxylation of glycine decomposition [20,24]. Li et al. [25] have also reported that the pH affected the kinetics of decarboxylation of alanine, which shows that the decarboxylation rate of the zwitterions form of amino acid is higher than that of the cationic and anionic forms. Our results also suggested that the ionic form of Asp also controls the reaction in high-temperature water.

There is no significant effect of pressure in the range of 10–30 MPa on the decomposition rate of Asp as shown in Fig. 7. The same result was observed in the hydrolysis of methyl propiolate ester [26] or decarboxylation of protein amino acids [27]. However, Li and Brill [28] reported that pressure has an obvious effect on the hydrolysis and cyclization of glycylglycine.

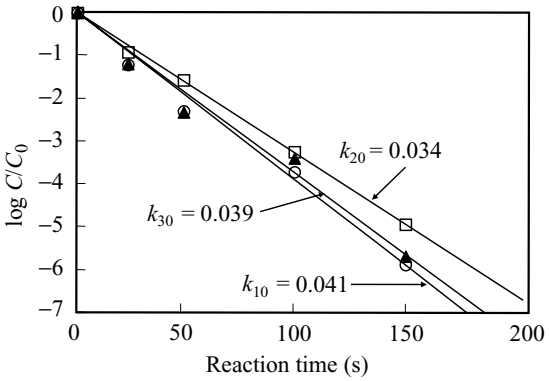

Figure 7 Effect of reaction pressure on decomposition rate of aspartic acid at 240°C and pH of 2.7 (○: 10 MPa, □: 20 MPa, and ▲: 30 MPa).

CONCLUSIONS

The decomposition of Asp under hydrothermal conditions was elucidated by studying the reaction kinetics and pathway. Deamination was the primary reaction that took place, evident from the significant amount of ammonia in the reaction products. Low-molecular weight carboxylic acids such as fumaric or maleic acid, pyruvic acid, and malic acid were also present in significant amounts. Traces of succinic acid, lactic acid, and Ala were also detected. Among the parameters investigated, temperature significantly affected the decomposition rate of Asp. The first-order reaction rate constant of 0.003 s^{-1} at 200°C dramatically increased to 0.231 s^{-1} at reaction temperature of 260°C. The activation energy was calculated to be about 144 kJ/mol using an Arrhenius equation. A slight effect of the changes in pH was evident, but no significant effect of pressure was observed at 240°C.

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