Threonine Transformation under Hydrothermal Conditions

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(Received October 18, 2004; CL-041218)

In order to revisit the kinetics of amino acid transformation in the chemical evolution, a series of hydrothermal experiments has been conducted for threonine (Thr) solution. Thr transformed into glycine (Gly) and other products including peptides and polymers at 121 to 160 °C. The Thr → Gly decomposition rate (k_1) is different from apparent Thr decreasing rate (k), because of the presence of other side reactions.

The emergence of the first life on the earth is the result of a long chemical evolution.[1,2] One of the important steps in the chemical evolution is a polymerization of organic matter. The polymerization of amino acids through oligomers to proteins is considered to have a thermodynamic difficulty.[3,4] That is, organic compounds cannot be polymerized spontaneously. One of the energy sources to overcome this difficulty is heat. However, the heating processes also cause the breaking down of amino acids and oligomers.[5–7] Thus it is important to evaluate the competition of the decomposition of starting materials against the polymerization. This study aims to evaluate the rates of amino acid decomposition processes by kinetic experiments on an OH-bearing amino acid (Threonine: Thr), often found in proteins of marine phytoplankton such as diatom.[8]

40 mL of the 0.1 mol/L L-threonine (Thr) aq solution (pH = 5.8 ± 0.1) was put in a PTFE (Teflon) container, which is placed in a screw-capped stainless outer vessel. Four sets of these reaction vessels were heated on the hot plate at 121, 131, 141, and 160 °C covered with aluminum foil for up to 7 days. All temperatures were measured with thermocouples inserted at the side of Teflon container. The temperature precision was within about ±4 °C and the accuracy of the heating duration is within about 4 h considering the warming periods to the desired temperature (50 to 220 min) and cooling periods after the heating (less than 3 h). After cooling at ambient condition, heated solutions were filtered with 0.45 μm-size disposable filter units and analyzed by high performance liquid chromatography (HPLC) system.

Dissolved organic compounds were analyzed by a HPLC apparatus (Jasco LC-Series) using AApak Na2–S2 column (Jasco Corp.) as described previously.[9] Fluorescence at 455 nm after post-column derivatization with phthalaldehyde was monitored as a function of the retention time. After heating, some of the solutions changed its color from colorless to brown while keeping their transparency. The film-like products were formed on the solution surface for some samples. All the solutions, which had film-like products, changed its color from colorless to brown.

HPLC analyses on the solution products showed that remaining Thr and glycine (Gly) are the major products (Figure 1). For higher temperatures and longer durations, glycylglycine (GlyGly), alanine (Ala), and some products were detected in trace amounts. Glycylglycylglycine was not detected in these conditions.

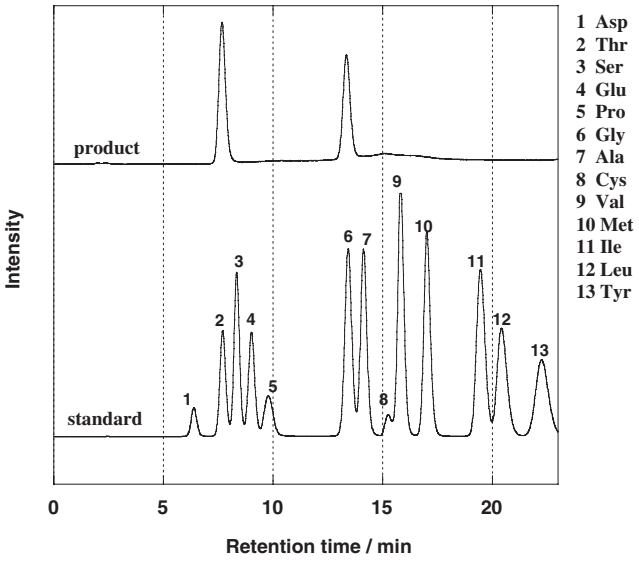

Figure 1. Typical chromatogram of the heated solution (500 times diluted).

At all the temperatures, Thr concentration decreased with time, while Gly concentrations increased. pH increased with time and heating temperatures (the highest pH was 9.3 ± 0.1). The decreasing rates of Thr and the increasing rates of Gly were faster for higher temperatures. Theses results suggest that the transformation of Thr into Gly occurs under these conditions. Vallentyne showed the nearly complete conversion of Thr to Gly during the course of pyrolysis in the absence of oxygen.[5] The Thr to Gly transformation observed here in the presence of oxygen is mostly consistent with the Vallentyne's results. However, the Gly yield was much less than was expected by the total conversion from Thr to Gly. Other products such as GlyGly, brown stuff in soluton and film-like products were formed in these conditions. Therefore, side reactions other than the Thr to Gly conversion are considered to be operating in our system. Characterization and quantification of other products such as GlyGly and film-like products will be discussed in a separate paper.

Based on the above arguments on reaction schema, the whole system of the Thr transformation reaction can be schematized as follows, with corresponding rate constants k_1 and k_2.

$$\mathrm{Thr} \begin{array}{l} \xrightarrow{k_1} \ \mathrm{Gly} \\ \xrightarrow{k_2} \ \mathrm{Others} \end{array}$$

It should be noted that Thr has both the decomposition (Thr → Gly) and the transformation (Thr → Others) pathways.

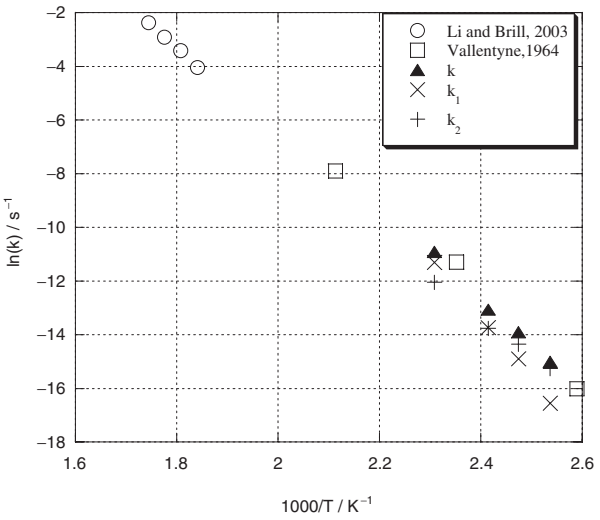

Figure 2. The Arrhenius diagram for the apparent decrease rate constants k of threonine together with the literature data.[5,10]

Let us now consider kinetic aspects of the Thr transformation reactions described as above with corresponding rate constants (k_1 and k_2). If we assume that, all of these reactions are regarded as the 1st order reaction, the following rate equations can be formulated.

$$[Thr] = [Thr]_0 e^{-kt} \quad (1)$$

$$k \equiv k_1 + k_2 \quad (2)$$

$$[Gly] = \frac{k_1}{k}([Thr]_0 - [Thr]) \quad (3)$$

[Thr] denotes the concentration of Thr in the product solution, $[Thr]_0$ the initial Thr concentration and [Gly] the Gly concentration.

In order to determine the rate constants from our experimental data sets, the following graphical method was used. The slopes for linear regression lines in natural logarithm of Thr concentration—time diagrams correspond to the first order Thr decreasing rate constants k ($= k_1 + k_2$). The obtained values are $3.0 \times 10^{-7}\,s^{-1}$, $9.2 \times 10^{-7}\,s^{-1}$, $2.1 \times 10^{-6}\,s^{-1}$, and $1.8 \times 10^{-5}\,s^{-1}$ at 121, 131, 141, and 160 °C, respectively. These apparent decrease rate constants k ($= k_1 + k_2$) of Thr were plotted in the Arrhenius diagram (Figure 2). These values are in good agreement with the Thr decrease rates reported previously.[5,10] The slope of the regression line in Figure 2 gives the apparent activation energy of 147 kJ/mol, in agreement with the values in literature data.[5,10]

Now, by using k, [Thr] and [Gly] values at each temperatures to the Eq 3, we can obtain k_1 values for each data points: the difference in values for the same temperature might be originated from the experimental errors in temperature and concentrations of Thr and Gly. Therefore the average k_1 values were determined at each temperature. The obtained average values of k_1 are $6.4 \times 10^{-8}\,s^{-1}$ (4.39×10^{-8} to $8.90 \times 10^{-8}\,s^{-1}$) at 121 °C, $3.4 \times 10^{-7}\,s^{-1}$ (1.31×10^{-7} to $4.42 \times 10^{-7}\,s^{-1}$) at 131 °C, $1.1 \times 10^{-6}\,s^{-1}$ (5.71×10^{-7} to $1.34 \times 10^{-6}\,s^{-1}$) at 141 °C and $1.2 \times 10^{-5}\,s^{-1}$ (1.18×10^{-5} to $1.27 \times 10^{-5}\,s^{-1}$) at 160 °C. Then we can calculate k_2 values by subtracting the above average k_1 values from k (Eq 2): $k_2 = k - k_1$. The obtained k_2 values are 2.4×10^{-7}, 5.8×10^{-7}, 1.0×10^{-6}, and $6.0 \times 10^{-6}\,s^{-1}$ at 121, 131, 141, and 160 °C, respectively.

In conclusion, the obtained apparent rate constants k ($= k_1 + k_2$) and the apparent activation energy (147 kJ/mol) showed a good agreement with the literature data for the decrease rate of Thr.[5,10] (Figure 2). However the k_1 and k_2 values showed different slopes in an Arrhenius diagram. These two slopes for k_1 and k_2 have a crossing points around 140 °C (Figure 2). At temperatures under 140 °C, the transformation reaction has larger rate constants (k_2) than the decomposition (k_1). This study demonstrated that the intrinsic amino acid decomposition rate such as that for Thr → Gly decomposition might be different from the apparent decrease rate of amino acids, because of the presence of other side reactions. This difference will be large at lower temperatures (Figure 2). Therefore, kinetic consideration on the chemical evolution of amino acids against their decomposition needs to be re-evaluated by taking into account the present results.

We would like to thank Dr. M. Ikoma for his discussions on the kinetic treatments of our data.

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Published on the web (Advance View) December 25, 2004; DOI 10.1246/cl.2005.158