Monitoring of Hydrothermal Reactions in 3 ms Using Fused-Silica Capillary Tubing

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(Received November 6, 1998; CL-980846)

Monitoring of hydrothermal reactions in the time range of 3 - 1000 ms was succeeded by flow reactor using fused-silica capillary tubing. The technique was applied for measuring the hydrolysis rate of ATP at 250 °C in which the minimum monitoring time was 3.1 ms. The rate constants of ATP hydrolysis was consistent with that was extrapolated of the Arrhenius plots in our previous study. This method enables milli-second monitoring of hydrothermal reactions.

Discovery of the catalytic activity of ribonucleic acids (RNA) have suggested that RNA played a central role in the first life on earth.[1] On the other hand, hydrothermal environment in the primitive ocean has been thought to significantly contribute to the emergence of life.[2] Recently, phylogenetic analysis has strongly suggested that the last common ancestor of all present organisms had nature of hyperthermophiles.[3] Thus, it is important to investigate the chemical evolution of RNA under hydrothermal environments. However, it seems to be surprising that the reactions of RNA in aqueous solutions at high temperatures have been less investigated. Although RNA is generally believed to be labile at high temperatures, quantitative data on the stability of RNA has not been available. One reason is that monitoring of fast reactions in aqueous solution at high temperatures was difficult or impossible. Recently, a new method was developed for monitoring of hydrothermal reactions in which the measurement of the rate of hydrolysis of adenosine 5'-triphosphate (ATP) was succeeded in the time range of 0.37 - 140 s at 125 - 300 °C.[4] The scope of this method has been investigated for monitoring faster reactions at higher temperatures. Consequently, a remarkable improvement has been achieved using fused-silica capillary tubing (FSCT) which allows monitoring the reactions in the time range 3 - 1000 ms at 250 °C.

The apparatus consists of a high pressure pump, a sample loop injector, a hydrothermal flow tube reactor which is controlled by cartridge heater, a cooling and back pressure coil, and a sampling port.[4] In this study, FSCT was mainly tested as tubing of the flow reactor, whereas stainless steel (SUS) tubing and teflon tubing were used in our previous study. The advantage of the use of FSCT is that a number of capillary tubing in different sizes of 0.005 - 0.1 mm inner diameter (ID) is commercially available. Further, it is also important that the inner wall is inert at high temperatures. In this study, minimum monitoring time was determined using a variety of FSCT in length and ID on the basis of analysis of the ATP hydrolysis.[4] Further, it was evaluated whether the inner surface of FSCT is inert on the ATP hydrolysis.

In this method, the velocity head loss limits the minimum value of residence time. Narrower size of FSCT allows monitoring of the reactions in shorter time period but the velocity head loss rises up inversely proportional to 4th power of ID under the constant volumetric flow rate. Fortunately, the velocity head loss decreases with increasing of temperature so that the velocity head loss is fairly reduced at higher temperatures. Consequently, the minimum size of FSCT which was capable to be used was 0.015 mm ID at 34 MPa, 250 °C.

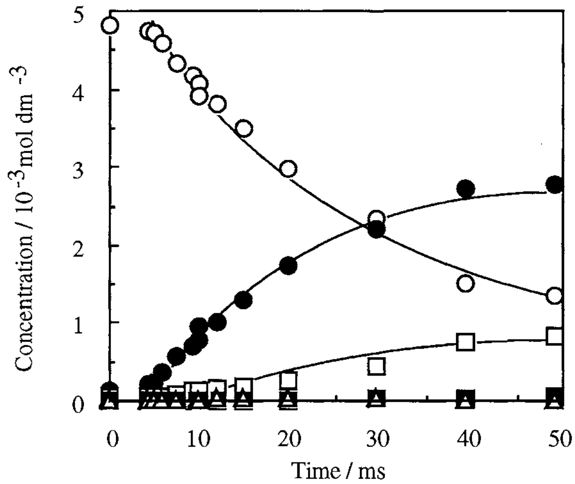

Figure 1. The reaction curves for the ATP hydrolysis using fused-silica capillary tubing.
[NaCl]=0.1 M (1 M = 1 mol dm^{-3}), [$MgCl_2$]=0.05 M, [Imidazole]=0.05 M, $[ATP]_{initial}$=0.005 M, pH=7.0, 250 °C, fused-silica capillary tubing : 0.025 mm (5 cm and 10 cm).
○: ATP, ●: ADP, □: AMP, ■: Adenosine, △: Adenine

On the other hand, fast monitoring requires quick heating up the sample solution. The heat transfer rate increases with decreasing of ID. The time required for heating up the sample solution (the heat up time) was evaluated on the basis of analysis of ATP hydrolysis rate. The heat up time was approximately 120 ms for 0.1 mm ID x 19 cm SUS, 40 ms for 0.05 mm ID x 19 cm FSCT, and 4 ms for 0.025 mm ID x 10 cm FSCT, and 2 ms for 0.015 mm ID x 5 cm FSCT. The heat up time decreased dramatically with decreasing of ID, then it results the reduction of the velocity head loss with decreasing of the length of tubing. Preheater has also been evaluated in order to decrease the heat up time. The reaction using the preheater at 215 °C did not give error to the reaction at 250 °C in which the heat up time was approximately 25 ms for 0.05 mm ID SUS tubing. Beside, the heat up time was also decreased with increasing of the length of FSCT ; the heat up time was about 50 ms for 0.05 mm ID x 38 cm SUS tubing and it was fairly reduced from that of 0.05 mm ID x 19 cm SUS tubing. The use of longer tubing was more convenient and efficient to reduce the heat up time than that of preheater.

The reaction curves using a 0.025 mm ID x 10 cm FSCT are shown in Figure 1. Very short heat up time was achieved and the plots demonstrate fine reproducible accuracy. The consecutive hydrolysis from ATP occurs in aqueous solution at high temperatures as given in the following equation.

$$\text{ATP} \longrightarrow \text{ADP} \longrightarrow \text{AMP} \longrightarrow \text{adenosine} \longrightarrow \text{Adenine} \longrightarrow \text{hypoxanthine}$$

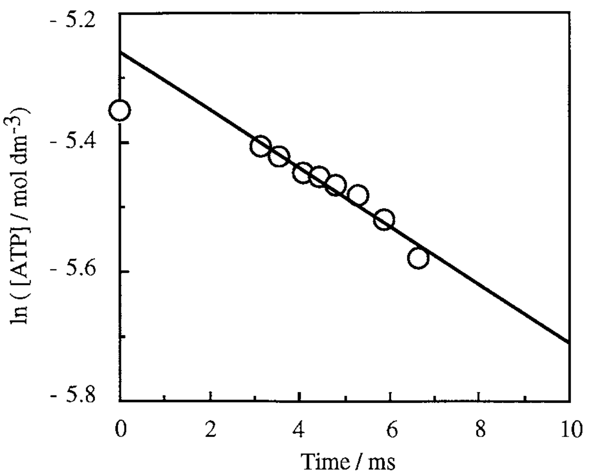

Figure 2. First-order rate plots for the disappearance of ATP. Fused-silica capillary tubing : 0.015 mm inner diameter x 5 cm All other conditions are the same as in Figure 1.

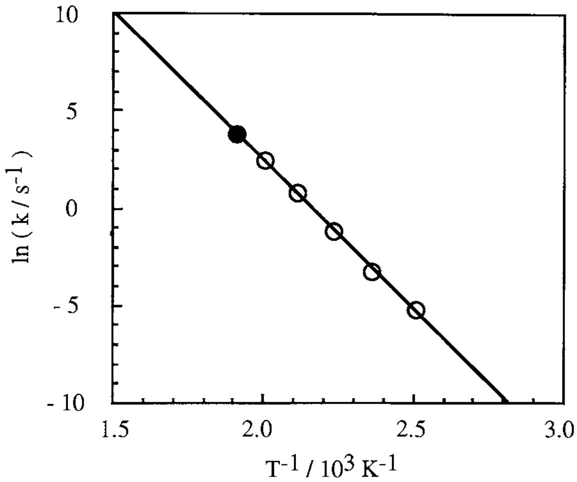

Figure 3. Arrhenius plots for the apparent rate constants of ATP hydrolysis.
○: Previous study in reference (4), ●: This study.

Although the consecutive hydrolysis of ATP at 250 °C was observable within 1 s using 0.1 SUS tubing, the observation of disappearance of ATP was not satisfactory. Beside, the loss of ATP was able to be monitored using 0.025 mm and 0.015 mm ID FSCT as the first-order plots are shown in Figure 2. The heat up time using 0.015 mm ID FSCT is remarkably shorter than that in other methods.[5,6]

The rate constant for the loss of ATP at temperatures 125 - 225 °C determined in our previous study and that at 250 °C determined in this study are shown in Figure 3. The rate constant at 250 °C are exactly on the line that was extrapolated of the Arrhenius plots at 125 - 225 °C. Thus, this result indicates that the monitoring at 250 °C using FSCT was excellently succeeded and the inner wall of FSCT is inert on the hydrolysis of ATP.

By this research, the most rapid monitoring method for hydrothermal reactions at 250 °C was achieved. This method enables monitoring of fast reactions on the chemical evolution of RNA and more general hydrothermal reactions, in principle.

Engineering Laboratory and Workshop Center, Osaka Prefecture University facilitated by generously preparing a part of the flow reactor. I thank Professor Taketoshi Nakahara in Osaka Prefecture University for the use of HPLC apparatus. This research was partially supported by the Inamori Foundation of Heisei 9 nendo.

References and Notes

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