Measurement of the rate of RNA hydrolysis in aqueous solution at elevated temperatures using a new monitoring method for hydrothermal reactions

Kunio Kawamura
Department of Applied Chemistry, College of Engineering, Osaka Prefecture University, Sakai, Osaka 599-8531, Japan

ABSTRACT
A new monitoring method for hydrothermal reactions, which is capable to monitor reactions in aqueous solution at 100 - 300 °C in 0.003 - 140 s, has been applied for the measurements of the rate of hydrolysis of oligonucleotides containing ribonucleic phosphodiester linkage. The hydrolyses of several types of oligonucleotides were monitored using the method at over 100 °C.

INTRODUCTION
Although the RNA world hypothesis is a most important theory on the scenario of how the earliest genetic information emerged on primitive earth (1), the relatively low stability of RNA under hydrothermal environments is a major stumbling block for this hypothesis (2). The reason is that it is assumed that the last common ancestor of all present organisms had nature of hyperthermophilus (3) and this seems to deduce that even the RNA world emerged at elevated temperatures. However, quantitative analysis of the formation and decomposition of RNA have less been carried out so that we have been investigating on kinetics of hydrolysis and formation of RNA under hydrothermal conditions (2,4). On the course of these studies, our group have recently developed a new monitoring method for rapid reactions in aqueous solutions at high temperatures (5,6), in which reaction curves can be obtained at 100 - 300 °C in the time range of 0.003 - 140 sec. In this report, monitoring of hydrolysis of oligonucleotides are demonstrated at elevated temperatures using this method. Further, investigations on chemical interactions of nucleotides have been tried.

EXPERIMENTAL
Oligonucleotides were purchased from GENSET (France) of HPLC purified grade. The hydrolysis of oligonucleotides was performed in aqueous solution containing 0.1 M NaCl, 0.1 M MgCl₂, 0.05 M imidazole (initial pH=8.0 at 25 °C), and ca. 1 x 10⁻⁵ M oligonucleotide at 150 - 200 °C. Polyetheretherketone (PEEK), and poly(tetrafluoroethylene) (PTFE) tubing was used for the flow tube reactor. Characterization and quantification of oligonucleotides were carried out by anion-exchange HPLC using DNA-NPR (TOSOH Co., Tokyo).

RESULTS AND DISCUSSION
The system for the measurements of nucleotide hydrolysis are based on the system described in previous studies (5,6). The residence time is able to be controlled by varying the flow rate (mL/min) and/or the inner volume of the flow tube reactor. We have designed an unique reaction system to analyze hydrolysis rate of phosphodiester linkage at elevated temperatures using oligonucleotides with sequence of 5'-GGCrCGGTTTTTCCGGCC-3' (oligol7) and 5'-GGCrCGGTTTTT-3' (oligoll). It is known that cleavage of phosphodiester bond of DNA is faster than those of RNA at low temperatures (7). Thus, it is expected that hydrolysis occurs firstly on phosphodiester linkage of ribose and then DNA units are hydrolyzed to monomers. The merit using these oligonucleotides is that hydrolysis of single position of phosphodiester bond of ribose can be detected, which is analyzed by a simple kinetic model. Further, influence and interactions of oligonucleotides with different sequence at elevated temperatures are possibly detected while no investigations of RNA have ever been carried out under hydrothermal conditions.

Hydrolysis of phosphodiester bonding at 150 - 200 °C was detected by HPLC in which GGCrCp and GGTGTTTCCGGCC formed from oligol7 and GGCrCp and GGTGTTT formed from oligoll11 as intermediates. Products were characterized by
assignment with authentic oligonucleotides (GGCCp, GGTTTTTCCGGCC, and GGTTTTT) (Figure). Basing on the characterization by HPLC, the hydrolysis is expressed by eq. (1) - eq. (2).

$$\text{GGCrCGGTTTTTCCGGCC}$$

$$\rightarrow \text{GGCrCp} + 5'\text{-GGTTTTTCCGGCC} \quad \text{(1)}$$

$$\rightarrow \text{Gp} + \text{Cp} + \text{Tp} \quad \text{(2)}$$

The results demonstrate exactly what was expected for oligo17 hydrolysis and a same type of reaction was observed for oligo11. Beside, the hydrolysis reaction rate using PEEK and PTFE tubing was in good agreement. Thus, it is concluded that there is no influence by tubing material for these reactions. The reaction mechanism of phosphodiester bonding have been extensively investigated with and without RNases. The results indicates that the cleavage of oligo17 and oligo11 at elevated temperatures obey the mechanism established at lower temperatures (8). The half-life times of oligo17 and oligo11 were 15 s and 4 s at 175 °C. This seems to indicate that there is difference on hydrolytic stability between the oligonucleotides even at elevated temperatures.

Actually, Tm values are fairly lower than temperatures tested in this study. Thus, the difference of the oligonucleotides is not understandable by conventional theory on the basis of Watson-Crick type hydrogen bonding and/or stacking among nucleotide bases. Further investigations are in progress.

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REFERENCES