Hydrolytic Stability of Ribose Phosphodiester Bonds within Several Oligonucleotides at High Temperatures Using a Real-Time Monitoring Method for Hydrothermal Reactions

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The hydrolytic stability of ribose phosphodiester bond within several types of oligonucleotides was measured at 150–200 °C using a real-time monitoring method. It was observed that the stability of a phosphodiester linkage is dependent on the surrounding sequences of the phosphodiester linkage at high temperatures. The fact indicates that some weak interactions, such as hydrophobic interaction, might be effective within the oligonucleotides at high temperatures.

Discovery of thermophilic organisms in association with hydrothermal conditions and its phylogenetic analyses support that life on earth might have originated in hydrothermal systems.[1] On the other hand, the low stability of RNA seems to be in conflict with the RNA world hypothesis.[2] However, it was difficult to measure the rate of RNA hydrolysis in real time over 100 °C since the degradation of RNA is so rapid. Thus, it has been necessary that the kinetic information at high temperatures is indirectly obtained from the temperature dependence of kinetic analyses below 100 °C, but actually there have been less studies on the temperature dependence. Thus, we recently developed a new monitoring method for hydrothermal reactions, in which it was established that the residence time of sample solutions exposed to high temperatures is accurately controlled within 2 ms to 150 s by rapid heating and quenching using a very narrow flow tube reactor.[3] In this study, real-time monitoring the hydrolysis of ribose phosphodiester bond with a –rC$^{3'}$pG– sequence was succeeded in several oligonucleotides at 150–200 °C.

The monitoring system for hydrothermal reactions was set up as described in previous studies,[3] in which polyetherketone tubing was used at 150–200 °C. For the comparison of kinetic data, hydrolyses at 65–80 °C were monitored using a batch method. Five types of oligonucleotides—5'-GGCrCGGTTTTTCCGGCC-3' (oligo-17), 5'-GGCrCGGTTTTT-3' (oligo-11), 5'-GGCCGGTTrCGTTCCGGCC-3' (oligo-18), 5'-CrCGG-3' (oligo-4), 5'-GGGCCrCGGGTTTTTCCCGGGCCC-3' (oligo-23), and dinucleotides rC$^{2'}$rG, rC$^{3'}$rG, dCdG—were tested. The melting temperatures (T_m) of oligo-17, -18, and -23 were determined using a CSC 6100 Nano II differential scanning calorimeter (DSC) (Calorimetry Sciences Corp., U.S.A.).

Five types of oligonucleotides involving –rC$^{3'}$pG– sequence were selected, since phosphodiester bond cleaves more rapidly than other DNA sequences as shown in eq 1.[4] Moreover, guanine and cytosine rich oligonucleotides are expected to result stronger intermolecular interaction than adenine and uracil rich oligonucleotides. Besides, imidazole was chosen to control pH since imidazole could have been formed prebiotically[5] and also has been frequently used as a model of the active site of histidine residue in ribonuclease.[6] HPLC of hydrolyzed oligo-17 (Figure 1) visualizes that the degradation started within a few seconds at 200 °C at the position of –rC$^{3'}$pG– sequence to be cleaved into two oligonucleotides, and then those were further degraded into monomer units. The hydrolytic degradation is expressed by eq 1 for the hydrolysis of oligo-17.

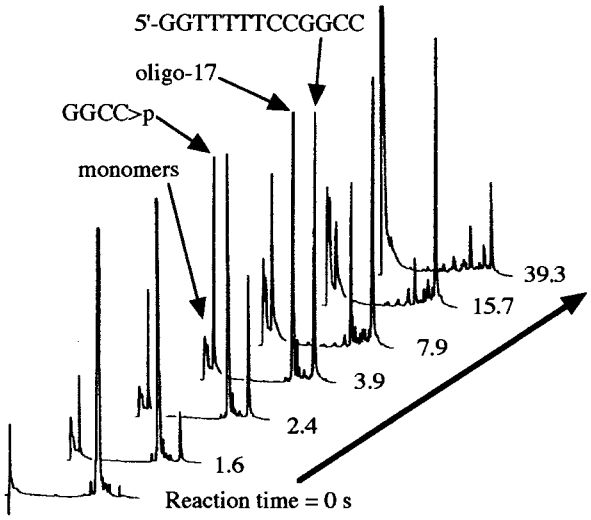

Figure 1. HPLC profiles during the hydrolysis of 5'-GGCrCGG-TTTTTCCGGCC-3' (oligo-17) at 200 °C. Reaction conditions : [NaCl] = 0.1 M, [$MgCl_2$] = 0.1 M, [imidazole] = 0.05 M, pH = 8.0. The reaction was performed using the monitoring system of hydrothermal reactions.

$$\text{5'-GGCrCGGTTTTTCCGGCC} \rightarrow \text{5'-GGCrC>p} + \text{5'-GGTTTTTCCGGCC} \quad (1)$$

The reaction curves for oligo-17 and oligo-11 are shown in Figure 2 and the apparent rate constants (k_{app}) were determined using a computer program SIMFIT (Table 1).[7]

Figure 2 and Table 1 indicate that lower stability of oligo-11 than other 4 types of oligonucleotides was detected at 150–200 °C. This finding is stimulating since the temperatures are much higher than T_m of oligonucleotides as described below,

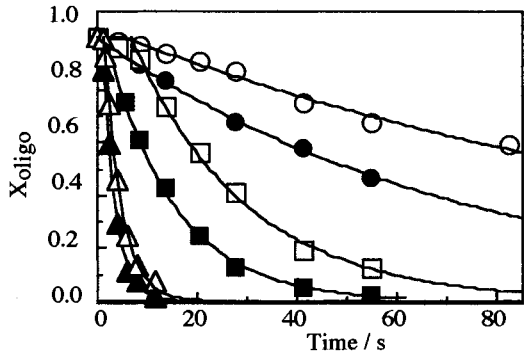

Figure 2. Reaction curves of the hydrolyses of oligo-17 and oligo-11 at 150–200 °C. Reaction conditions are the same as shown in Figure 1. X_{oligo} indicates the ratio of remaining ribose phosphodiester bond. ○, □, △: oligo-17; ●, ■, ▲: oligo-11; temperatures (°C): ○, ●:150; □, ■: 175; △, ▲: 200. The lines drawn through the experimental points were fitted by SIMFIT.

Table 1. The apparent rate constants (k_{app} / s^{-1}) of the hydrolysis of nucleotides at 65–200 °C

T / °C	65	80	150	175	200
nucleotides					
oligo-17	$(4.85 \pm 0.08) \times 10^{-7}$	$(2.42 \pm 0.09) \times 10^{-5}$	$(7.15 \pm 0.12) \times 10^{-3}$	$(4.33 \pm 0.05) \times 10^{-2}$	$(2.92 \pm 0.04) \times 10^{-1}$
oligo-11	$(2.30 \pm 0.04) \times 10^{-5}$	$(4.47 \pm 0.06) \times 10^{-5}$	$(1.35 \pm 0.14) \times 10^{-2}$	$(7.30 \pm 0.06) \times 10^{-2}$	$(3.90 \pm 0.06) \times 10^{-1}$
oligo-4	$(1.02 \pm 0.02) \times 10^{-5}$	$(2.61 \pm 0.04) \times 10^{-5}$	$(1.01 \pm 0.01) \times 10^{-3}$	$(4.68 \pm 0.07) \times 10^{-2}$	$(2.21 \pm 0.02) \times 10^{-1}$
oligo-18	$(6.26 \pm 0.29) \times 10^{-6}$	$(2.57 \pm 0.06) \times 10^{-5}$	$(1.06 \pm 0.02) \times 10^{-2}$	$(4.89 \pm 0.18) \times 10^{-2}$	$(2.69 \pm 0.06) \times 10^{-1}$
oligo-23	$(6.61 \pm 0.30) \times 10^{-7}$	$(6.74 \pm 0.18) \times 10^{-6}$	$(1.09 \pm 0.04) \times 10^{-3}$	$(5.44 \pm 0.07) \times 10^{-2}$	$(2.92 \pm 0.04) \times 10^{-1}$
$rC^{3'}rG$	$(3.67 \pm 0.14) \times 10^{-6}$	$(8.13 \pm 0.04) \times 10^{-6}$	$(1.27 \pm 0.02) \times 10^{-3}$	$(6.18 \pm 0.04) \times 10^{-3}$	$(3.22 \pm 0.02) \times 10^{-2}$
$rC^{2'}rG$	$(3.73 \pm 0.17) \times 10^{-6}$	$(7.57 \pm 0.07) \times 10^{-6}$	$(1.10 \pm 0.01) \times 10^{-3}$	$(4.91 \pm 0.03) \times 10^{-3}$	$(2.44 \pm 0.02) \times 10^{-2}$
dCdG	NA[a]	$(1.02 \pm 0.06) \times 10^{-7}$	$(2.04 \pm 0.24) \times 10^{-4}$	$(2.03 \pm 0.02) \times 10^{-3}$	$(1.73 \pm 0.02) \times 10^{-2}$

[a]Not analyzed.

and this fact may reflect some weak interactions at high temperatures. For example, at low temperatures, the phosphodiester bonds within double helix are more stable than those in single-strand regions,[8] and the stability of phosphodiester bond is somewhat dependent on the position within hairpin loops.[9] However, it seems that the dependence of k_{app} on surrounding sequences at high temperatures is substantially different from that at low temperatures, so it is difficult to understand from the conventional Watson–Crick model. For example, surrounding sequences might interact with –$rC^{3'}G$– linkage. Moreover, the predominant pathway is the imidazole-catalyzed hydrolysis, where imidazole promotes the deproponation of ribose hydroxy group,[6] the interaction between oligonucleotide and imidazole might contribute. The values of k_{app} of oligo-17 increased to $(10.3 \pm 0.17) \times 10^{-3}$ s^{-1} at 150 °C and $(4.86 \pm 0.06) \times 10^{-2}$ s^{-1} at 175 °C with addition of 0.01 M L-histidine, so this might reflect the difference of interactions of imidazole and L-histidine approaching oligo-17. The mechanism of the hydrolytic cleavage has been extensively investigated below 100 °C,[6,8–10] so detail analysis at high temperatures is being in progress.

The Arrhenius plots showed good linearity at 150–200 °C and the apparent activation energy (E_{app}) was 107 kJ mol^{-1} (oligo-17), 104 (oligo-11), 109 (oligo-23), 100 (oligo-4), 105 (oligo-18), 90 ($rC^{3'}rG$), 87 ($rC^{2'}rG$), and 139 (dCdG). This fact indicates that the values of k_{app} at 150–200 °C are consistent with those at 65–80 °C.[3] Thus, the mechanism deduced at low temperatures can be applied to high temperatures, where the transphosphorylation in phosphodiester bond is regarded as the rate-determining step reflecting E_{app}.[6] The similar E_{app} values of 5 types oligonucleotides suggests that the difference of k_{app} is mainly due to changes in entropy. Besides, the values of k_{app} of oligo-23 at 65 and 80 °C, and oligo-17 at 65 °C are notably smaller than other oligonucleotides, in which the stability of oligo-17 and oligo-23 were 10–47 times greater than oligo-4, -11, and -18 at 65 °C. The inhibition of hydrolysis of oligo-23 and oligo-17 at 65–80 °C is assumed to be due to the formation of hairpin structure[6] since oligo-23 and -17 could form hairpin at below T_m, but oligo-4 and -11 could not. To confirm this assumption, the values of T_m of oligo-23, -18, and -17 were determined with DSC. The values of T_m decreased in the order 85.2 °C (oligo-23) > 76.9 °C (oligo-18) > 76.0 °C (oligo-17) and the fact is consistent with the enhancement of the hydrolytic stability of oligo-23 and oligo-17 at 65–80 °C. This evaluation suggests that the extrapolation of the kinetic data at low temperatures to high temperatures should be careful.

Besides, it is interesting that the stability of $rC^{2'}rG$ becomes somewhat greater than $rC^{3'}rG$ at 150–200 °C (Table 1), while it seems that large difference of the stability of 3',5'- and 2',5'-linkages was only observed within a helical RNA.[9–11] Furthermore, it is surprising that the magnitude of k_{app} of $rC^{2'}rG$ and $rC^{3'}rG$ is notably smaller than that of other oligonucleotides at 150–200 °C. The 2'-OH group on 3'-terminal guanosine of $rC^{2'}rG$ and $rC^{3'}rG$, which is absent in the 5 types of oligonucleotides, may inhibit the cleavage of phosphodiester bond. Moreover, the stability of phosphodiester bond of dCdG is much higher than ribose phosphodiester bonds at low temperatures, but becomes comparable at 200 °C.

Conclusively, in this study the hydrolytic stability of oligonucleotides was directly evaluated at high temperatures using the real-time monitoring method and the dependence of the hydrolytic stability was detected on oligonucleotide sequences and between 2',5'- and 3',5'-linkages. The analyses are principally impossible from the extrapolation of kinetic data obtained at low temperatures using conventional techniques, so details are currently being investigated.

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