Condensation reaction of hexanucleotides containing guanine and cytosine with water soluble carbodiimide

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ABSTRACT
The condensation reactions of hexanucleotides involving guanine and cytosine in the presence of water-soluble carbodiimide (WSC) have been investigated as a model reaction of the prebiotic formation of RNA under primitive earth. The reactions formed cyclic hexanucleotides and dimers in which the product yields were dependent on the sequence.

INTRODUCTION
It is widely believed that RNA molecules played a central role in the first life on earth (1,2). If this hypothesis is correct then, RNA or RNA-like molecules formed spontaneously under primitive earth reaction conditions. There have been a number of successful studies of the condensation reactions using activated nucleotides in the presence and absence of RNA template (3-8). Examples include the condensation of the 2-methylimidazolide of 5'-GMP on a poly(C) template (3-5), the condensation reaction of the 5'-phosphorimidazolides of nucleotides in the presence of metal catalysts (6), and that in the presence of clay minerals (7,8). Moreover, kinetic investigations of the condensation reaction of short oligonucleotides using WSC have been performed to understand the minimal replicating systems (9,10).

However, the pathway from the spontaneous formation to the replication system of oligonucleotides is still unclear. In this paper, the condensation reaction of the hexanucleotides containing guanine and cytosine bases was investigated at 0 °C in the presence of WSC.

EXPERIMENTAL
Oligonucleotides with sequences of 5'-pGGGCCrC (oligo-6-1), 5'-pGCGCGrC (oligo-6-2), and pGCCGrG (oligo-6-3) were purchased from GENSET (France) of HPLC purified grade. The condensation reactions of the oligonucleotides, which are regarded as the monomer unit, were performed in an aqueous solution containing NaCl, MgCl₂, imidazole, and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (WSC), and 1 x 10⁻⁴ M oligonucleotide at pH 8.0 and 0 °C. The reaction was monitored for 7 days and the products were analyzed by anion-exchange HPLC using DNA-NPR (TOSOH Co., Tokyo).

RESULTS AND DISCUSSION
Two main peaks (product-1 and product-2) were observed on the anion-exchange HPLC for the reaction products of the oligo-6-1, oligo-6-2, and oligo-6-3. The retention time of product-1 was shorter than that of product-2 and the extent of product-1 was higher than that of product-2. The condensation reaction did not proceed in the absence of imidazole or WSC. It is reasonable to consider that the imidazolide formed on the 5'-terminal phosphate of oligo-6 under the reaction condition (9-12). Additionally, it was confirmed that WSC is stable for 7 days at 0 °C from the absorbance at 230 nm. These results are consistent with pervious studies on the
condensation reactions of oligonucleotide with WSC. Thus, it is regarded that the condensation reactions of oligo-6 occurs in the presence of WSC.

The retention time of the product-2 of oligo-6 on the anion-exchange HPLC was the same as that of pGCCCGGGCCGGrG (oligo-12). Moreover, the enzymatic hydrolysis of the products using RnaseT2 indicates that both product-1 and product-2 involve 3’-5’-phosphodiester linkage in the oligonucleotides. Basing on the analysis, it is assigned that product-2 is the dimer of oligo-6 and product-1 is the cyclic hexanucleotide.

The yields of the product-1 and product-2 were not notably influenced with the concentration of salts and oligo-6, while those were increased with increasing the WSC concentration. The yields of product-1 and -2 in a solution containing 0.2 M NaCl, 0.075 M MgCl2, 0.1 M imidazole, 0.2 M WSC (pH=8.0, 0 °C, 7 days) were 50.4 % (product-1), 5.3 % (product-2) for oligo-6-1, 53.4 % (product-1), 1.8 % (product-20 for oligo-6-2, and 29.5 % (product-1), 8.3 % (product-2) for oligo-6-3. This fact indicates that the reaction rate and the product yields are dependent on the sequence of the hexanucleotides. The difference of the reaction rate may be due to that phosphodiester bond forms between G-C sequence for oligo-6-1 and oligo-6-2 while that forms between G-G sequence for oligo-6-3. Besides, the higher yield of the dimer formation in oligo-6-3 may be explained by the model shown in Scheme 1.

\[
\begin{array}{cccccccccccc}
\text{H} & \text{O} & \text{H} & \text{G} & \text{C} & \text{C} & \text{C} & \text{G} \\
\text{G} & \text{C} & \text{C} & \text{G} & \text{G} & \text{C} & \text{C} & \text{G} & \text{G} \\
\end{array}
\]

The apparent rate constants were determined from the pseudo-first order rate plots of the disappearance of oligo-6 and were decreased in the order of oligo-6-1 (1.65 x 10^{-6} s^{-1}) ≈ oligo-6-2 (1.64 x 10^{-6}) > oligo-6-3 (8.90 x 10^{-7}). The reaction rate has roughly similar magnitude to other types of the spontaneous formation of RNA (3, 9, 10, 12).

These results indicate that the formation of oligonucleotides under prebiotic conditions is dependent on its sequence. Further, the rapid formation of the cyclic oligonucleotide is interesting since cyclic DNA is used as the present bacterial gene.

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REFERENCES