STUDIES OF THE NON-ENZYMATIC TEMPLATE-DIRECTED SYNTHESIS OF OLIGO-GUANYLATES

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The elucidation of the mechanism by which a polymeric nucleic acid molecule becomes transcribed or replicated is of fundamental importance in the study of the origin of life. As shown by Orgel and coworkers, polycytidilate will facilitate the template-directed synthesis of the complementary oligomer (oligoguanylate) from 2-MeImpG. Although the conditions used are not realistically prebiotic and, so far the process is only efficient in going from poly(C) to oligo(G), a study of this reaction was undertaken in order to confirm and extend this work, and increase our understanding of the processes of transcription and replication at the molecular level.

In a typical experiment the method of Inoue and Orgel (1982), with some modifications, was followed. First the 2-methylimidazolide of guanosine 5'-monophosphate was prepared according to Joyce et al. (1984). Second, the reaction of this activated mononucleotide in the presence of polycytidylate was carried out for 10 or more days at 0°C in an incubation mixture containing 2,6 lutidine, NaCL and $MgCl_2$. Third, Na_4EDTA was used to stop the reaction at the particular point of interest. Tris-HCl was added in order to decrease the pH of the reaction until neutrality was reached. Pancreatic ribonuclease A was added to hydrolyze the template, poly(C), without affecting the newly formed oligonucleotide (oligo G). The termination of the oligomerization process is dependent on a set of changes brought about by Na_4EDTA, Tris-HCl and pancreatic ribonuclease A. Fourth, the products were separated and analyzed by HPLC using a stainless steel column (0.4cm x 50cm) with a particle size of 44μm. The column was packed with RPC-5 polymer powder in the perchlorate form.

Some 40 experiments were carried out, one half using 2-MeImpG and the other half using ImpG. The concentration of reactants, Mg^{++}, and pH were the major variables. Several chromatograms were obtained for reactions carried out under different conditions of pH. Figure I shows a typical chromatogram of the oligo(G) synthesis at pH 7.6. Careful analysis of the chromatograms obtained demonstrate a decrease of the reaction rate as the pH is increased from 7.6 to 8.2. The dependence of the yield of oligomers on the concentration of Mg^{2+} varies with the pH. At a pH lower than 7 the reaction exhibits a triple helix character.

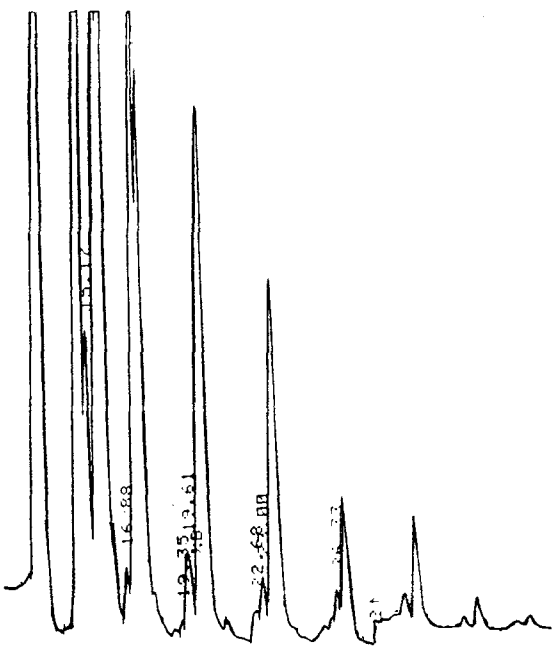

Fig. 1. HPLC elution profile of oligoguanylates obtained in Exp. 22.

The double helix, poly(C)-growing oligo(G) reaction at pH 7.6-8.2 is partially dependent on the Mg^{2+} concentration. As the Mg^{2+} concentration is increased, the length of the newly synthesized oligonucleotides becomes extended.

The minor peaks with slightly shorter retention times than the major products almost certainly correspond to oligomers that contain one or a very small number of 2'-5'links. These peaks increase in size relative to the major peaks as the pH increases from 7.6 to 8.2. This is possibly due to the occasional ionization of G residues in the preformed oligo(G) strand at high pH values which interferes with the regularity of the poly(C):oligo (G) double helix and reduces the regiospecificity of the condensation reaction. 2'-5'linkages increase upon a longer incubation time.

The system under consideration has produced oligonucleotides up to 10 mer with the prevalence of 3'-5' phosphodiester linkages. Longer oligomers may be obtained through sliding or ligation. The sliding mechanism was suggested some time ago (Orô and Sherwood, 1974) and has been shown to occur under certain conditions (Chen et al., 1985). Ligation may be used to unite two or more oligomers of similar (or variable) lengths to produce a longer oligomer. The union of the two oligomers would have to take place without disturbing the overall three dimensional conformation of the oligonucleotides. The mechanistic approach of this process will probably involve end to end joining of the 3' hydroxyl group of one oligomer with the 5' phosphate group of another oligomer.

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