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KINETICS OF THE TEMPLATE-DIRECTED OLIGOMERIZATION OF GUANOSINE 5'-PHOSPHATE-2-METHYLIMIDAZOLIDE: EFFECT OF TEMPERATURE ON INDIVIDUAL STEPS OF REACTION

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Non-enzymatic, template-directed reactions have been proposed as models for prebiological polynucleotide synthesis (Orgel, 1986). Chemically activated mononucleotides react in the presence of a polynucleotide, acting as the template in a Watson-Crick base-pairing fashion, and form the complementary daughter-polynucleotide. Phosphoimidazolide-activated nucleotides have been used successfully as substrates in these reactions (Inoue and Orgel, 1983).

We studied the kinetics of the guanosine 5'-monophosphate-2-methylimidazolide (2-MeImpG) reaction in aqueous pH 8.0 solutions in the presence and in the absence of polycytidylate (poly(C)), acting as the template at 6°C, 23°C and 37°C. In the absence of the template the major reaction pathway of 2-MeImpG is hydrolysis of the P-N bond to form the unreactive guanosine 5'-monophosphate (5'-GMP) and 2-methylimidazole. Concentrated solution of 2-MeImpG (>0.02 M) in the absence of the template form only small amount of dinucleotide, $(pG)_2$, but in the presence of poly(C), oligoguanylates, $(pG)_n$ with $2 \leq n \leq 40$, can be detected (Inoue and Orgel, 1982).

We were able to determine the rate constants for individual steps of this reaction. Our conclusions can be summarized as follows: (i) The effect of the template on the rate of the internucleotide bond formation, which corresponds to an approximately 200-fold catalysis (obtained from k_n/k_2 where k_n the biomolecular rate constant of reaction of an oligomer with $n \geq 5$ and k_2 the rate constant of dimerization of 2-MeImpG in the absence of poly(C) stays constant in the temperature range of 6°C to 37°C. This implies similar activation energies for the two reactions, i.e. the dimerization in the absence of the template and the elongation in the presence of the template. This result may suggest that the template acts as a site for bringing the reactants together without affecting the transition state of the internucleotide bond formation (not a true catalyst). (ii) In the early stages of the reaction hydrolysis of 2-MeImpG, initiation of the oligomerization (k_2, k_3 and k_4) and elongation (k_n, $5 \leq n \leq 11$), occur simultaneously and compete in such a way to give rise to a larger total percentage of shorter oligomers ($n<5$) at the lower temperature. This competition implies that for a constant supply of activated monomer the oligomerization process would favor longer oligomers at the higher temperature.