J Mol Evol (1995) 41:169–173

JOURNAL OF MOLECULAR EVOLUTION

Faster Rates with Less Catalyst in Template-Directed Reactions

Anastassia Kanavarioti, Eldon E. Baird

Department of Chemistry and Biochemistry, University of California, Santa Cruz, CA 95064, USA

Received: 19 December 1994 / Accepted: 23 February 1995

Abstract. We have recently shown that the polycytidylic acid-directed polymerization of guanosine 5′-monophosphate 2-methylimidazolide (2-MeImpG) is amenable to kinetic study and that rate determinations as a function of 2-MeImpG concentration can reveal much mechanistic detail (Kanavarioti et al. 1993). Here we report kinetic data which show that, once the reaction has been initiated by the formation of dimers, the elongation of dimers to form longer oligomers is accelerated by *decreasing* polycytidylate (poly(C)) concentration from 0.05 to 0.002 M. This result is consistent with the previously proposed mechanism. The increase in the observed pseudo-first order rate constant for formation of the trimer, k_3', and the corresponding constant for formation of oligomers longer than the trimer, k_i' (k_i' is independent of oligomer length for $i \geq 4$), with decreasing template concentration for a given monomer concentration is attributed to an increase in template *occupancy* as template concentration is reduced.

Key words: Polycytidylic acid — Oligoguanylate synthesis — Chemical evolution

Introduction

Template-directed, nonenzymatic, reactions are still the only plausible pathway for the formation of biopolymers on prebiotic Earth (Kanavarioti 1994). In practice, however, the applicability of these reactions to the synthesis of nucleic acids is severely limited. It is hoped, though, that a better mechanistic understanding of the chemistry involved will lead to the discovery of conditions that make these reactions more efficient in the synthesis of long, informational polymers. In an earlier report (Kanavarioti et al. 1993), we showed the feasibility of a thorough kinetic study by determining rate constants for each step of the poly(C)-directed polymerization of 2-MeImpG (Inoue and Orgel 1982). We have also measured the binding of 2-MeImpG on poly(C) under the conditions of the polymerization reaction (Kanavarioti et al. 1995).

According to our previous kinetic study, oligoguanylate synthesis is best described by a reaction scheme [Eq (1) and Kanavarioti et al. 1993] that consists of a series of consecutive steps, each representing the formation of an oligomer of length i by reaction of the one of length i-1 with

$$\mathrm{M} \xrightarrow{k_2'} \mathrm{G_2} \xrightarrow{k_3'} \mathrm{G_3} \xrightarrow{k_4'} \mathrm{G_4} \rightarrow \rightarrow \mathrm{G_{i-1}} \xrightarrow{k_i'} \mathrm{G_i} \xrightarrow{k_{i+1}'} \mathrm{G_{i+1}} \quad (1)$$

2-MeImpG. In Eq. (1), M stands for the monomer 2-MeImpG, while G_2, $G_3 \ldots G_{i+1}$ are oligomers of length 2, 3 ... $i + 1$, with no distinction made for possible isomers. It is implied that the reacting monomers are template-bound and associated at the 3′-end of a growing oligomer. k_2', $k_3' \ldots k_i'$ are the formal pseudo-first-order rate constants, defined by Eqs. (2) and (3).

$$d[G_2]/dt = k_2'[\mathrm{M}] - k_3'[\mathrm{G_2}] \quad (2)$$

$$d[\mathrm{G_i}]/dt = k_i'[\mathrm{G_{i-1}}] - k'_{i+1}[\mathrm{G_i}] \quad (3)$$

Correspondence to: A. Kanavarioti

In the cited study poly(C) concentration was kept constant at 0.05 M and 2-MeImpG concentration varied in the range 0.005 M to 0.045 M. The following conclusions were reached. The values of k_i' ($i \geq 4$) for the formation of the 4-mer and longer oligomers are independent of length and somewhat higher than k_3' which, in turn, is much higher than k_2'. The complex dependence of k_2', k_3', and k_i' ($i \geq 4$) on [2-MeImpG] is the result of a combination of two factors: the cooperative nature of the binding of the monomer to the template, and the requirement that the growing end of an oligomer be complexed with at least three monomers (k_3' and k_i' ($i \geq 4$)), or that dimerization (k_2') occurs within a complex of at least six monomers.

In the present paper, we report kinetic data from reactions using a fixed 2-MeImpG concentration and poly(C) concentration that varied from being much less to being much larger than the 2-MeImpG concentration. The kinetic analysis indicates, that once the reaction has been initiated by the formation of dimers, the elongation of dimers to form longer oligomers is accelerated by *decreasing* the poly(C) concentration within the range studied.

Materials and Methods

All the materials and procedures employed in this study have been described in detail in Kanavarioti et al. (1993). *N*-(2-hydroxyethyl)piperazine-*N'*-2-ethanesulfonic acid (HEPES) was purchased from Aldrich. Analysis of the samples was performed with a 1090 Hewlett Packard liquid chromatograph on an RPC5 column. The 2-MeImpG preparation used here had less than 1% guanosine 5'-monophosphate (5'GMP) and less than 0.8% diguanosine 5',5'-pyrophosphate (GppG) impurities as tested with C_{18} chromatography. In the HPLC profiles, the 5'GMP peak was not resolved well enough to estimate the observed area; the area corresponding to 5'GMP was calculated based on the injection volume, observed oligomer area, and a pH 12 calibration parameter of 3.05 pmol 5'GMP/HPLC unit.

Calculation of Occupancy and Amount of Template-Bound Monomer. For the calculation of occupancy (θ) and amount of template-bound monomer ($[M]_T$) the stochiometry of the complex needs to be known. Poly(C) forms 1:1 complexes with guanosine derivatives at pH > 7 and 2:1 complexes at pH < 7 (Howard et al. 1964; Davies and Davidson 1971). Evidence that under our conditions at pH 8, poly(C) forms a 1:1 complex with 2-MeImpG comes from the binding isotherm (Kanavarioti et al. 1995) and from the experiments with [2-MeImpG] > or ≫ [poly(C)] ran to completion. For example, the yield of oligoguanylates with 0.002 M poly(C) and 0.02 M monomer is approximately 0.002 M, expressed in guanosine equivalents, whereas with 0.03 M template and 0.04 M monomer the yield is about 0.03 M. These high yields are only consistent with a 1:1 complex and not with a 2C: 1G complex, mainly because at the temperature of incubation the oligoguanylates do not dissociate from the template and there is no turnover of the reaction.

The occupancy, θ, for a 1:1 complex is given by Eq. (4) where poly(C) concentration is expressed in cytidine equivalents. The parameters obtained in the preceding paper allow the determination of θ for all the experimental mixtures tested in this study by means of Eq. (5) (Cantor and Schimmel 1980) and the mass balance Eq. (6)

$$\theta = \frac{[M]_T}{[\text{poly(C)}]} \tag{4}$$

$$\theta = \frac{(Q[M]^{\alpha_H}}{1 + (Q[M])^{\alpha_H}} \tag{5}$$

$$[M]_{tot} = [M]_T + [M] \tag{6}$$

In Eq. (5), Q is the equilibrium constant for association of 2-MeImpG to a site of poly(C) adjacent to an already associated monomer or oligomer, with Q presumed to be independent of the length of the stack. [M] is the concentration of free monomer in solution, α_H, the Hill constant (see Cantor and Schimmel 1980), is a measure of the cooperativity of the association with $\alpha_H = \sqrt{Q/q} \cdot q$ represents the equilibrium constant for association of 2-MeImpG to an isolated site of poly(C). In Eq. (6), $[M]_{tot}$ is the total monomer concentration. $Q = 180$ M^{-1} and $q = 2.22$ M^{-1} were determined from the binding isotherm at 23°C for [poly(C)] ranging from 1.78–7.55 mM (see Kanavarioti et al. 1995). Assuming that the binding isotherm remains valid at poly(C) concentrations higher than 7.55 mM, the determined values of q and Q can be used to calculate θ and $[M]_T$ for any given set of $[M]_{tot}$/[poly (C)]. In practice, this was done as a spreadsheet calculation with Microsoft Excel on a Mac IIci by varying [M] by a few percent of a mM at a time and calculating θ, $[M]_T$ and $[M]_{tot}$ for a given [poly(C)] using Eqs. (5) and (6). The experimental values of $[M]_{tot}$ were then matched to calculated ones and the corresponding θ and $[M]_T$ for a certain [poly (C)] was read out from the spreadsheet. θ and $[M]_T$ are included in Table 1.

Results and Discussion

Reactions were carried out in a 0.5 M HEPES aqueous buffer at pH 7.95 ± 0.05 in the presence of 1.2 M NaCl and 0.2 M $MgCl_2$ at 23 ± 0.1°C; these are the same conditions used in Kanavarioti et al. (1993, 1995). Reactions were monitored for only a few hours so that no more than 15% of monomer was consumed, ensuring that the oligoguanylate products always remain the minor component of the system. Preparation and analysis of the samples as well as rate determinations were performed as described in detail in Kanavarioti et al. (1993). Table 1 summarizes the obtained kinetic parameters. This table also includes values for the occupancy (i.e., the fraction of template sites occupied by the monomer, θ, and the amount of template-bound monomer, $[M]_T$, obtained as described under Methods).

The effect of changing the poly(C) concentration on the polymerization is best illustrated in Fig. 1. It shows HPLC profiles of the product distribution of the reaction of 0.02 M 2-MeImpG in the presence of 0.05 M, 0.02 M, or 0.002 M poly(C) after approximately 3.5 h. It is seen that in the presence of 0.05 M poly(C) oligoguanylates up to 10- (top), with 0.02 M template up to 14- (middle) and with 0.002 M template up to 17 U long are being formed (bottom). In other words, during the same incubation time, the mixture with the least amount of template

Table 1. Summary of pseudo-first-order rate constants for oligomer formation (Eq. 1)

[poly(C)][a] M	$[M]_{tot}$[b] M	θ[c]	k_3'[d] h^{-1}	k_i'[d] h^{-1}	$10^4 \times k_2'$[d] h^{-1}	$10^3 \times [M]_T$[e] M
0.05	0.005	0.026	—	0.22	2.1	1.3
0.02	0.005	0.049	—	0.32	—	0.98
0.01	0.005	0.078	—	0.42	0.7	0.78
0.05	0.02	0.30	0.54	1.09	41	15.0
0.02	0.02	0.70	0.73	1.53	30	14.0
0.002	0.02	1.0	—	(2.36)[f]	(12)[f]	2.0
0.03	0.03	0.79	0.84	1.42	63	23.7
0.05	0.04	0.68	0.87	1.53	138	34.0
0.03	0.04	1.0	1.13	1.66	98	30.0

[a] [poly(C)] in C monomer equivalents. Kinetic parameters for the experiments with 0.05 M poly(C) are taken from Kanavarioti et al. (1993)

[b] M = 2-MeImpG; $[M]_{tot}$ as weighed out

[c] Occupancy, θ, is the fraction of template positions occupied by monomer obtained (see under Methods) using the association constants determined ($Q = 180\ M^{-1}$ and $q = 2.22\ M^{-1}$) from the binding isotherm of Kanavarioti et al. (1995)

[d] Pseudo-first-order rate constants determined as described in Kanavarioti et al. (1993); see also Eqs. (2) and (3). Accuracy of the rate constants is estimated at ±10–20%, although with 0.002 M poly(C) the accuracy is closer to ±25% because of the smaller yield of this reaction compared to the rest

[e] $[M]_T$ is the template-bound monomer obtained as described under Methods

[f] Perhaps overestimated due to technical difficulties; the k_i' value not included in Fig. 2

yielded the longest oligomer products, although the total yield of polymeric products, measured in terms of monomers incorporated into oligomers, decreases with decreasing poly(C) concentration (compare *y*-scale in Fig. 1). Rate determinations (Table 1 reports values of k_3' and k_i' ($i \geq 4$) as a function of [poly(C)] confirm these observations. Trends similar to the one for the experiment at 0.02 M monomer are exhibited by the data at 0.005 M and 0.04 M monomer (see Table 1).

How can we understand our results according to which the least amount of template leads to the longest oligomers? Let us briefly review what is presently known about this template-directed reaction. G mononucleotides, such as 2-MeImpG, cooperatively associate with the template by hydrogen bonding between G and C, and by stacking among the G units (Howard et al. 1964; Davies and Davidson 1971; Kanavarioti et al. 1995). When monomer concentration is much higher than the concentration of the template sites, the template is virtually saturated and polymerization is at its most efficient. Presumably, there is none or very little preference for a monomer to associate with a cytidine-site at the end of a stack of monomers or at the 3′-end of a preformed dimer or oligomer. It is then for statistical reasons that, on a partially occupied template, some of the initially formed dimers and subsequently some of the oligomers will not have monomers associated at their 3′-end and therefore the overall elongation process will be slowed down. It is important to realize that although fast equilibration allows the monomer to associate with any preformed oligomer, as well as with any stack of monomers, at low template occupancy there will always be oligomers that have none or not enough monomers associated at the 3′-end. The observation that three or more monomers are necessary for elongation to occur at optimal rate (Kanavarioti et al. 1993)[1] makes it understandable that even in cases where occupancy is significant, k_i' is not at maximum value (e.g., compare 4th and 5th row in Table 1: both k_3' and k_i' values are depressed with $\theta = 0.3$ as compared to $\theta = 0.7$ for the same amount of monomer (0.02 M)). Based on the above reasoning, there should be a correlation between the degree of saturation of the template sites and the observed values of k_3' and k_i'. Figure 2 indicates a curvilinear dependence of k_3' and k_i' on θ, consistent with the views expressed above. It is self-evident why the elongation rate constants level off when approaching saturation.

The corresponding rate constant for dimer formation, k_2', does not follow the trend exhibited by k_3' and k_i'. Instead, the k_2' values increase strongly with [2-MeImpG] and correlate, albeit nonlinearly, with the concentration of template-bound monomer, $[M]_T$ (Table 1 and Fig. 3). The nonlinear dependence is consistent with the proposed mechanism in which dimerization occurs in long stacks of monomers (Kanavarioti et al. 1993). Since k_2' is responsible for the overall yield of the polymerization (every oligomer was once a dimer), it is not surprising that the yields diminish substantially with 0.002 M template ($[M]_T = 0.002$ M here) as compared to 0.02 and 0.05 M (compare *y*-axes in Fig. 1).

The fact that k_2' correlates with $[M]_T$ whereas k_3' and k_i' correlate with occupancy can be attributed to an im-

[1] Similar effects of catalysis by additional associated monomers besides the reacting one is observed with guanosine monomers (Wu and Orgel 1992a) and with cytidine monomers in the template-directed elongation of hairpin oligonucleotides (Wu and Orgel 1992b).

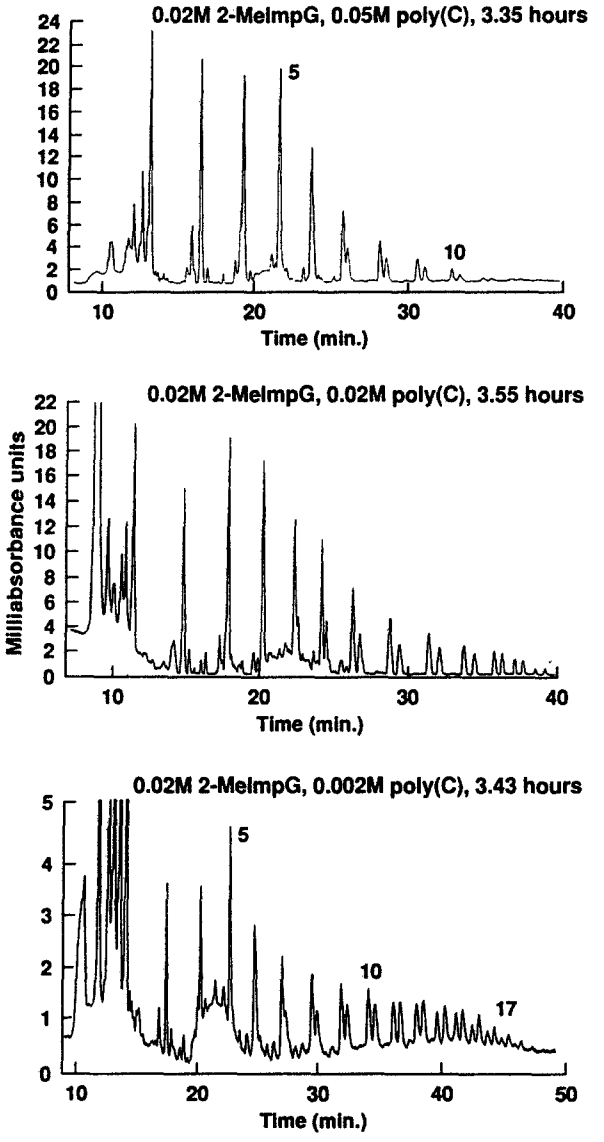

Fig. 1. High-performance liquid chromatography profiles: effect of template concentration on yield and length of oligomeric products in the polymerization of 0.02 M 2-MeImpG (5, 10, and 17 stand for 5-, 10-, and 17 unit long G-oligomer, respectively). For each oligomer length, there are more than one isomer that appear as bunches of peaks. Samples were treated in the exact same way in order to make the profiles directly comparable. Oligomers of the same length do not always have the same retention time. Retention times vary substantially with the age of the RPC5 column.

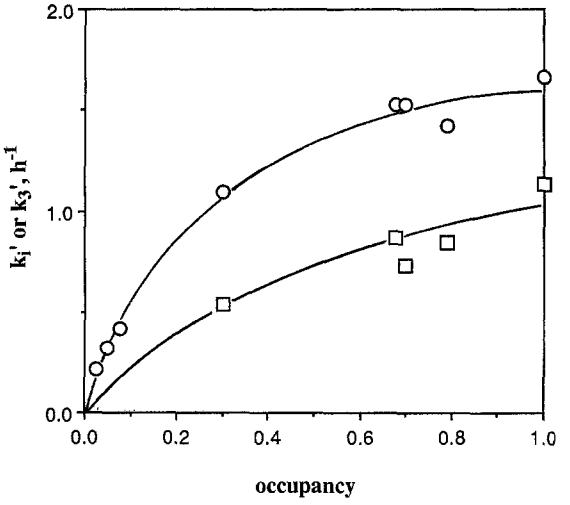

Fig. 2. Pseudo-first-order rate constants for formation of oligoguanylates, trimer (k_3', *squares*) or longer than trimer (k_i', *circles*), plotted vs occupancy. Occupancy, θ, is the fraction of template positions occupied by monomer. The value of k_i' with 0.002 M poly(C) not included here (see Table 1).

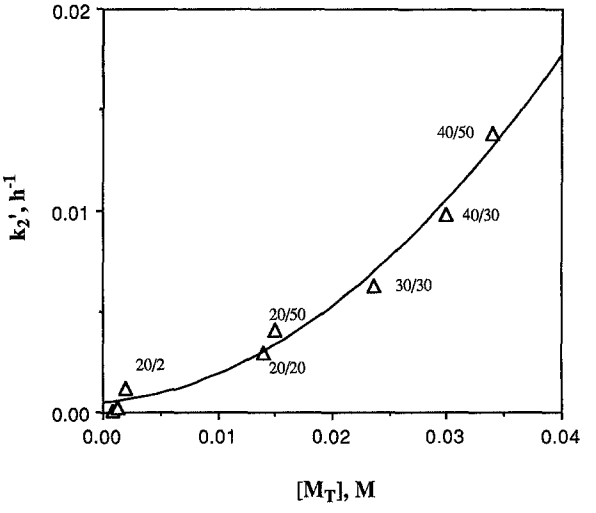

Fig. 3. Pseudo-first-order rate constant for dimerization, k_2', as a function of template-bound monomer, $[M]_T$ (see Table 1). Experiments identified as $[2\text{-MeImpG}]_{tot}/[poly(C)]$ in mM expressed as monomer equivalents. The two unlabeled points correspond to 5/10, and 5/50, for 0.005 M monomer with 0.01 and 0.05 M template, respectively.

portant difference between the two types of processes. Dimerization (k_2') is a process that occurs everywhere on the template between two neighboring template-bound monomers as long as the requirements with respect to the length of a stack have been met. In contrast, elongation (k_3' and k_i') is a process that occurs only at the 3′-end of a preformed oligomer and depends on the probability that an oligomer, which is the minor component of the system as studied, will have the appropriate number of monomers associated with it. This probability increases with occupancy and this is why elongation correlates with θ.

Although the correlations presented in this work lead to a reasonable understanding of the experimental system under study, they certainly do not describe it quantitatively. We are presently collecting a larger set of data than the one reported in Table 1 with the objective to test and perhaps refine the quantitative model presented in Kanavarioti et al. (1993).

Implications for Chemical Evolution

It is perhaps not the rate difference in the elongation between a half-saturated, let us say, and a saturated template that is worth discussing within a prebiotic context,

but the realization that in the presence of a smaller amount of template the polymerization yields the longest possible oligomers. The latter is a welcome exception to the notion that prebiotic chemistry requires implausibly high concentrations of starting materials (Shapiro 1984). Our observation that less poly(C) can lead to longer oligoguanylate products is an example where less is better, at least if length and not yield, is a measure of complexity and progress in chemical evolution.

Acknowledgments. We thank NASA's Exobiology program for support of this work through grant NCC 2-534, Prof. Claude F. Bernasconi of the University of California at Santa Cruz, and Dr. S. Chang of NASA Ames Research Center for providing the facilities and advice.

References

Cantor CR, Schimmel PR (1980) Biophysical chemistry Freeman and Co, San Francisco, Part III, p 864 and references therein and in Kanavarioti et al. (1995) Affinity of guanosine derivatives for polycytidylate revisited. J Mol Evol 41:161–168

Davies RJH, Davidson N (1971) Base pairing equilibria between polynucleotides and complementary monomers. Biopolymers 10:1455–1479

Howard FB, Frazier J, Lipsett MN, Miles HT (1964) Infrared demonstration of two- and three-strand helix formation between poly(C) and guanosine mononucleotides and oligonucleotides. Biochem Biophys Res Comm 17:93–102

Inoue T, Orgel LE (1982) Oligomerization of (guanosine 5′-phosphor)-2-methylimidazolide on poly(C). J Mol Biol 162:201–218

Kanavarioti A (1994) Template-directed chemistry and the origins of the RNA world. Origins Life 24:479–494

Kanavarioti A, Bernasconi CF, Alberas DJ, Baird EE (1993) Kinetic dissection of individual steps in the poly(C)-directed oligoguanylate synthesis from guanosine 5′-monophosphate 2-methylimidazolide. J Am Chem Soc 115:8537–8546

Kanavarioti A, Hurley TB, Baird EE (1995) Affinity of guanosine derivatives for polycytidylate revisited. J Mol Evol 41:000–000

Shapiro R (1984) The improbability of prebiotic nucleic acid synthesis. Origins Life 14:565–570

Wu T, Orgel LE (1992a) Nonenzymatic template-directed synthesis on oligodeoxycytidylate sequences in hairpin oligonucleotides. J Am Chem Soc 114:318–322

Wu T, Orgel LE (1992b) Non-enymatic template-directed synthesis on hairpin oligonucleotides. 2. Templates containing cytidine and guanosine residues. J Am Chem Soc 114:5496–5501