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DNA-Catalyzed Polymerization†

Xiaoyu Li, Zheng-Yun J. Zhan, Rachel Knipe, and David G. Lynn*

Departments of Chemistry and Biology, Emory University, Emerson Hall, Atlanta, Georgia 30322

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As early as 1956, inspired by the Watson−Crick double helix,[1] synthetic polymers were explored as templates to direct the course of polymerization reactions.[2] Investigations of biological macromolecule templates appeared 10 years later,[3] yet the ability to direct polymerization along a template and read sequence and chain-length information specifically in the absence of biological catalysts has remained limited. For example, DNA-catalyzed phosphodiester formation has been investigated intensively,[3,4] and succeeds with activated guanine ribonucleotides (Gp) and oligodeoxycytidine $(dCp)_n$ templates. $(dAp)_n$, $(dTp)_n$, $(dGp)_n$, and mixed sequence templates, however, are poor catalysts. Even the successful $(dCp)_{10}$ template gives a distribution of products, from GpG dimers to $(Gp)_8$ octamers, but no decamer products are detected, and chain length specificity has not been realized.[4]

Here we report nonphosphodiester-based reaction conditions that allow sequence- and chain-length-specific reading of a DNA template. Replacing phosphodiester formation with reductive amination, a reaction exploiting preequilibrium imine formation on the template, the octameric template $(dAp)_8$ catalyzes quantitative reduction of the thymidine monomer 5′-H_2N-dT-3′-CH_2CHO (T) to the octameric polyamine product. Figure 1 shows the time course for this reaction, where dimer and tetramer products appear only as intermediates.[5] Therefore, this process enables the first chain-length-specific reading of a DNA template via monomer polymerization,[5b] and the reaction follows classical step-growth kinetics, distinctly different from that reported with phosphodiester formation.

The generality of step-growth kinetics was further investigated with the synthetic dimer, 5′-H_2N-dT_NT-3′-CH_2CHO (T_NT), where the N subscript indicates an amide linkage joining the modified thymidine nucleosides. This substrate was converted cleanly to the octamer product with $(dAp)_8$ template catalysis (Figure 2). Only intermediate tetramer appeared, confirming the step-growth kinetics, and again achieving precise chain-length control.

The reaction was designed to proceed via intermediate template-bound imine, and the ^{15}N-labeled substrates 5′-$H_2N-T_NT_NT$-3′-CH_2CHO $(T_N)_3$ and 5′-$H_2N-T_NT_NT_NT$-3′-CH_2CHO $(T_N)_4$ were prepared with labels at each linking nitrogen atom to enable imine detection via $^1H-^{15}N$ HMQC NMR analyses.[6] Both substrates displayed thermal melting with their respective $(dAp)_8$ and $(dAp)_6$ DNA complements,[6b] and imine formation was readily confirmed at temperatures below the melting temperature. For example at 10 °C, 5′-amine and amide signals were observed at 9.5 and 99.6 ppm for $(T_N)_3$. Upon addition of the complementary $(dAp)_6$ template, three isomeric $^1J_{(N-H)}$ protonated imine signals appeared at 199.9/6.88, 200.9/7.65, and 205.7/8.45 ppm; tentatively assigned as geometric imines in slow exchange, the most downfield signal assigned as the more abundant protonated trans imine isomer.[6c] Clean ligation products were obtained with reduction at higher

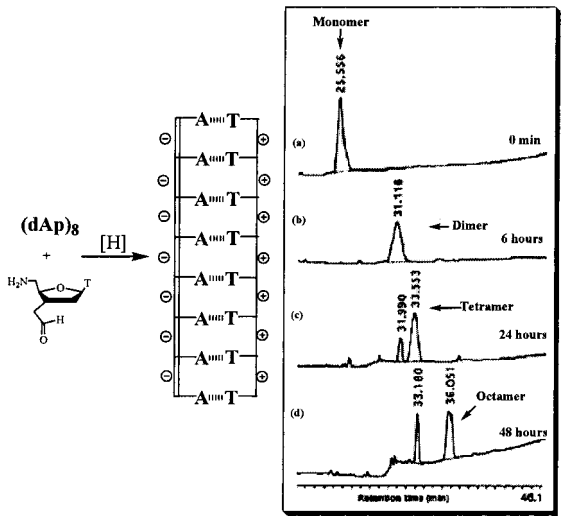

Figure 1. HPLC analysis of template-directed polymerization of (T) by $(dA_p)_8$.[5] (a) time zero; (b) 6 h; (c) 24 h; (d) 48 h. MALDI-TOF (THAP/citrate): dimer, dT_N-NH-T_N, *m/z* 519.8631 $(M + H)^+$, calcd $C_{24}H_{34}N_6O_7$ 518.2489 (M^+); tetramer, dT_N-$(NH\text{-}T_N)_3$, *m/z* 1021.8128 $(M + H)^+$, calcd $C_{48}H_{68}N_{12}O_{13}$ (M^+); 1020.5029; octamer dT_N-$(NH\text{-}T_N)_7$, *m/z* 2026.0110 $(M + H)^+$, calcd $C_{96}H_{136}N_{24}O_{25}$, 2025.0109 (M^+). Template eluted at ∼13min.

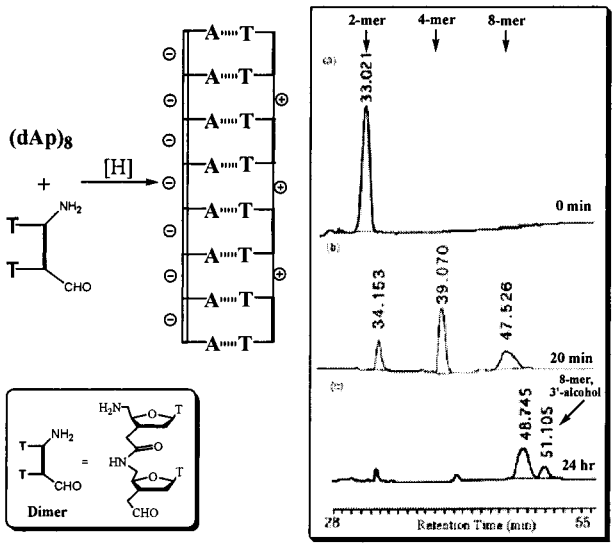

Figure 2. HPLC analysis of template-directed polymerization of (T_NT) by $(dA_p)_8$.[5] (a) time zero; (b) 20 min; (c) 24 h, 8-mer was partially reduced to 3′-CH_2OH after 24 h. MALDI-TOF (THAP/citrate): octamer T_NT-$(NH\text{-}T_NT)_3$-CHO, *m/z* 2082.51 $(M + H)^+$, calcd $C_{96}H_{128}N_{24}O_{29}$, 2080.9279 (M^+); T_NT-$(NH\text{-}T_NT)_3$-CH_2OH, *m/z* 2083.47 $(M + H)^+$, calcd $C_{96}H_{130}N_{24}O_{29}$, 2082.9436 (M^+). Template eluted at ∼13min.

* Author for correspondence. Telephone: (404) 727-9348. Fax: (404) 727-6586. E-mail: dlynn2@emory.edu.

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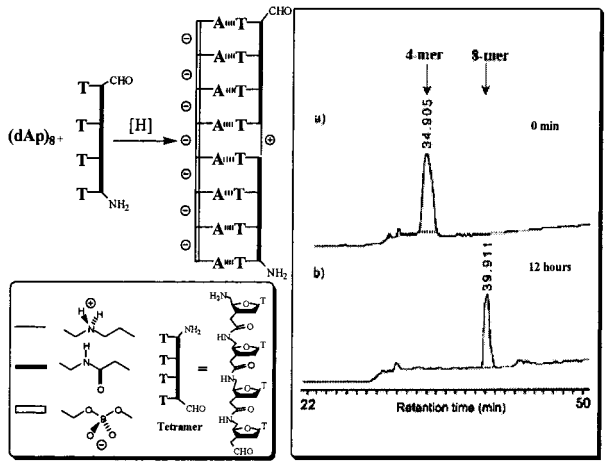

Figure 3. Template-directed ligation of $(dT_N)_4$ by $(dAp)_8$:[5] (a) time zero; (b) reaction complete after 12 h. MALDI-TOF (THAP/citrate): octamer product, 5′-d$(T_N)_4$-NH-$(T_N)_3$-3′-CH_2CHO, *m*/*z* 2109.65 $(M + H)^+$, calcd $C_{96}H_{124}N_{24}O_{31}$, 2108.8864. Template eluted at ∼13min.

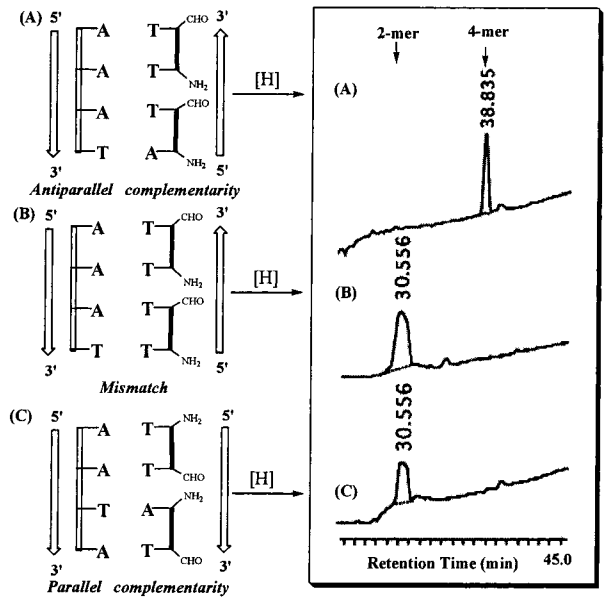

Figure 4. HPLC analysis of template-directed catalysis[5] with template d(ApApApT) of (A) (T_NA) and (T_NT) after 24 h, (B) (T_NT) after 24 h, and (C) template d(ApApTpA) with (T_NA) and $(T_N)_2$ after 24 h. MALDI-TOF: 4-mer, dA_NT-NH-T_NT, *m*/*z* 1058.51 $(M + H)^+$, calc. $C_{48}H_{63}N_{15}O_{13}$, 1057.4730 (M^+). Templates eluted at 12−14min.

temperatures. For example, $(T_N)_4$ was quantitatively reduced to the octamer amino aldehyde at ambient temperatures, but only in the presence of the $(dAp)_8$ template, and again no higher oligomers could be detected (Figure 3).

Sequence selectivity in the reaction was investigated with the heterodimer 5′-H_2N-A_NT-3′-CH_2CHO (T_NA) substrate. The template 5′-dApApApT-3′ catalyzed the ligation of a mixture of (T_NA) and (T_NT) substrates (Figure 4A), but no product was detected with either (T_NA) or (T_NT) substrates alone (Figure 4B). Therefore HPLC analyses place a lower limit on selectivity; the reaction selects against a single (A/T) mismatch by >100:1 in a single ligation. Likewise, the 5′-dApApTpA-3′ template does not serve as catalyst for (T_NA) and (T_NT) ligation, consistent with the modified-sugar backbone stereoselectively maintaining exclusive antiparallel complementarity in the reaction (Figure 4C).

Step-growth kinetics are expected for template-directed polymerization catalysts employing substrates with reactive ends. Polymerization rates should be a function of both substrate/template binding affinity and the number of template binding sites. A $(dAp)_n$ template that forms preferential antiparallel complexes has $(n - 1)$ unique (T) binding sites that contribute to the overall imine concentration in the first ligation steps. When comparing $(dAp)_8$ with $(dAp)_4$ templates, $(dAp)_8$ requires 2-fold more (T) to complement the template, but due to the additional binding sites, the relative imine concentration is expected to increase by almost 40-fold, significantly enhancing initiation rates. This number continues to grow with template length following the expression $(n - 1)(n/2)^2$. As polymerization proceeds, the number of binding sites falls, but product/template affinity increases to enhance the later coupling steps. Balancing the rates of these steps is expected to be critical for controlling reaction fidelity along longer templates.

The ability to read a DNA template sequence and chain length specifically represents a critical extension of biology's template-directed syntheses, represented by its Central Dogma. The step-growth kinetics outlined here are clearly different than the chain-growth processes selected in biology, and it should be possible to exploit strengths and limitations of other synthetic reactions for reading biopolymers.[7] Knowing that amine-linked DNAs are catalytically active phosphodiester ligases[8] suggests that these materials may be further extended to access synthetic replication and selection strategies. Therefore, the accurate synthetic translation of information encoded in biological macromolecules, exploiting more standard polymerization reactions, should enable the preparation of a diversity of monodisperse, sequence-specific functional materials for synthetic biology.

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Supporting Information Available: Details of substrate syntheses (PDF). This material is available free of charge at http://pubs.acs.org.

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(5) (a) General reductive amination conditions and product analysis. Templates, 2 mM.; substrate, 4 mM for tetramer, 8 mM for dimer, 16 mM for monomer; 20 equiv of $NaBH_3CN$ was added at room temperature. The reaction mixtures were analyzed by reverse phase HPLC: Rainin HPXL, Phenomenex Prodigy 5 analytical ODS(2) C18; Rainin Dynamax UV detector at 260 nm, and eluted with MeOH in H_2O: 0%−5% from 0 to 8 min, 5%−20% from 8 to 9 min, 20%−35% from 9 to 24 min, 35%−60% from 24 to 25 min, 60%−100% from 25 to 50 min. (b) With addition of 10 equiv more of $NaBH_3CN$ after 48 h, remaining tetramer was converted to octamer.
(6) (a) Zhan, Z.-Y. J.; Lynn, D. G. *J. Am. Chem. Soc.* **1997**, *119*, 12420. (b) Thermal melting experiments were performed at 5 mM in D_2O by ^1H NMR. T_M of $(T_N)_4$, 20 °C, $(T_N)_3$, ∼10 °C. (c) ^1H chemical shifts were referenced to DSS, ^{15}N was referenced to $^{15}NH_4NO_3$, and samples were dissolved in 90% H_2O and 10% D_2O; Experiments were performed on a Varian Unity 400 NMR spectrometer; the $^1J_{(N,H)}$ was optimized at 90 Hz.
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