Template-free, polymerase-free DNA polymerization†

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A combination of a DNA ligase and a restriction endonuclease provides a DNA polymerase activity, which might suggest a novel strategy for polymer synthesis.

This paper reports a strategy of enzymatic DNA polymerization, which requires neither templates nor DNA polymerases, instead, where a DNA ligase and a restriction endonuclease act alternatively. The polymerization mechanism superficially resembles that of the natural DNA polymerization.[1] The DNA ligase adds DNA monomers to active sites of a growing DNA chain, and the restriction endonuclease removes extra parts from the ligated monomers and regenerates active sites. DNA chains can be elongated by at least 230 basepairs (bp) in this strategy.

DNA polymerization is one of the most important biological processes. In the cell, it is catalyzed by a family of delicate enzymes, namely DNA polymerases. DNA polymerases synthesize DNA molecules by replication of template DNA molecules, which starts from short nucleotides primer.[1] In special cases, DNA polymerases such as a telomerase can synthesize DNA without external DNA templates. The telomerases contain intrinsic RNA templates.[2] Elongation needs nucleotide triphosphate (NTP) substrates. A DNA chain incorporates one nucleotide monophosphate (NMP) in each step and generates a pyrophosphate by-product.[1] The natural strategy is elegant and efficient, and can synthesize very long DNA molecules. Usually, DNA polymerases are complicated protein complexes that contain many subunits to ensure their proper functions. Such polymerases are the result of a long history of natural evolution. However, it is not clear how the evolution of DNA polymerases starts? In this paper, we have demonstrated that a combination of two simple enzymes (a DNA ligase[3] and a restriction endonuclease) can mimic the primary function of a DNA polymerase: catalyzing the formation of DNA polymers by chain polymerization.

Fig. 1 illustrates the polymerization strategy. Both primer and monomer molecules have hairpin structures. Each has a double-stranded duplex stem, a single-stranded T4 loop, and a 3 base-long single-stranded overhang (sticky end). The two sticky ends are complementary to each other, and ensure that the primer and the monomer can recognize and associate with each other through Watson–Crick base-pairing. Addition of a DNA ligase can covalently join these two molecules into one single molecule, which has a dumbbell shape: a duplex capped by T4 loops at both ends. Upon ligation, a complete bipartite restriction endonuclease (Bgl I)[4] recognition site will be generated at the boundary between the primer and the monomer. The Bgl I recognition sequence is originally split into two halves. One is on the primer and the other on the monomer. Only when covalently joined can the bipartite site be recognized and cleaved by the Bgl I enzyme. After restriction digestion, the dumbbell molecule becomes two segments. The cleavage site is 5 bp away relative to the original ligation site. Thus the primer elongates by 5 bp in a ligation–cleavage circle. Bgl I digestion generates a 3-base long sticky end. In the current design, the DNA sequence is chosen in such a way that the new generated sticky end is identical to the sticky end on the original primer molecule. The resulting molecule will grow longer and longer as the ligation–cleavage process repeats. This overall process is similar to natural enzymatic DNA polymerization, except the DNA chain grows in steps of five basepairs instead of one base.

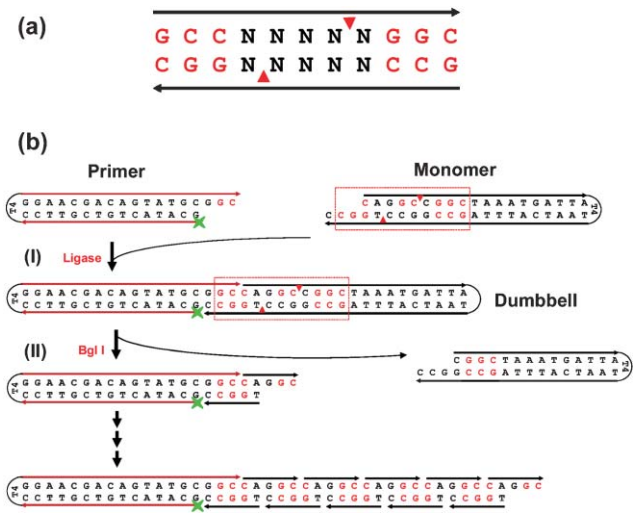

Fig. 1 Scheme of the DNA polymerization. (a) The Bgl I recognition sequence. While both outside ending three basepairs are fixed, the central five basepairs are variable. The cleavage sites are indicated by the arrowheads. The arrows on the lines indicate the DNA strand polarity from 5′ to 3′. (b) The design of the DNA primer and monomers. The polymerization process contains two steps: (I) Stick-ends cohesion and ligation: a primer and a monomer recognize and associate with each other to form a doubly nicked dumbbell structure, and a DNA T4 ligase seals the nicks; (II) Restriction: Bgl I restriction endonuclease cleaves the dumbbell molecule and regenerates the original sticky end of the primer. Repetition of steps (I) and (II) leads to DNA polymerization. Although step (II) can go in the reverse direction catalyzed by DNA ligases, the overall process is irreversible because step (I) is irreversible. Note the complete Bgl I sites are boxed with a dashed line. The red colored letters indicate the Bgl I recognition sequences. The green stars indicate the positions of the radioactive isotopes for monitoring the polymerization.

This strategy works well as designed. We first mixed primer and monomer at a molar ratio of 1:20. After addition of Bgl I and T4

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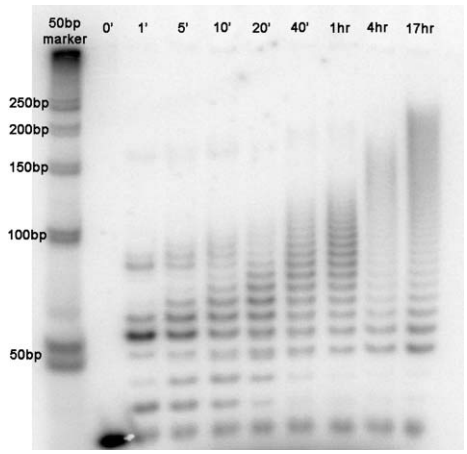

Fig. 2 Time course of DNA polymerization analyzed by native polyacrylamide gel electrophoresis (PAGE). The reaction times are indicated at the top of the gel image. The far left lane contains a 50 bp ladder, which consists of a series of DNA duplex size markers. Note the primer strand is labeled with radioactive isotope ^{32}P and DNA molecules are visible only if they contain ^{32}P labels.

DNA ligase, this mixture was incubated at 22 °C. This temperature is a compromise between the restriction enzyme and the ligase. The optimal working temperatures are 37 °C and 16 °C for Bgl I and T4 DNA ligase, respectively.[3,4] Aliquots of the reaction mixture were withdrawn at different times and quenched with excess EDTA. All samples were analyzed by native polyacrylamide gel electrophoresis (PAGE). The primer strand was labeled with ^{32}P, a radioactive isotope (indicated as green stars in Fig. 1). Fig. 2 shows the image of the gel. DNA molecules are visible only if they contain ^{32}P labels. Incubation as short as one minute leads to clearly observable polymerization. The molecular sizes of the DNA molecules continuously increase in the course of the experiment duration (17 hours). The longest molecules have reached about 250 basepairs (bp) long, which is 235 bp longer than the original primer molecules (15 bp). In other words, the primer has been elongated by 47 monomers (5 bp).

We have further examined the temperature effect on the polymerization process. The above mentioned experiment has been repeated at four different temperatures: 4 °C, 16 °C, 22 °C, and 37 °C (Fig. 3). As the temperature increases in this range, the overall polymerization process accelerates. We attributed the acceleration to the increased Bgl I activity. At lower temperature, Bgl I works far away from its optimal activity. The dumbbell DNA molecules accumulate, which correspond to the dark bands (indicated by black dots) shown in the gel, and retard the overall process. When the temperature increases, the dumbbell DNA molecules are digested effectively and the overall process speeds up. Accordingly, the bands corresponding to the dumbbell molecules disappeared at a high temperature. The speed and effectiveness of the DNA polymerization goes up in the order of 4 °C, 16 °C, 22 °C, and 37 °C. The size distribution shifts to higher molecular weight from 4 °C to 37 °C constantly.

It would be reasonable to expect that the DNA polymerization could be much more efficient after fine tuning reaction factors, such as primer/monomer ratio, temperature, buffer composition, and balancing the ligase/endonuclease activities. However, such optimization is beyond the scope of this work. Here we focus on showing the possibility of reconstituting the DNA polymerase from enzymes with simpler functions. We expect that this strategy would be useful in DNA nanotechnology.[5] For instance, this strategy could be used to prepare novel DNA nanostructures, such as modifying existed DNA nanostructures by programmable extension of DNA duplexes. This strategy would also be expected to be useful in the field of DNA nanomachines.[6] Indeed, a loosely related strategy has been used in a protein-driven DNA device.[6f]

In summary, we have shown a successful mimicry of DNA polymerases by combining a DNA ligase and a restriction nuclease. This study shows that a polymerase is essentially a combination of a ligase (to attach a monomer precursor to a growing chain) and a cleavase (to remove the extra portion of the monomer precursor and regenerate the active site). Note that there is no evidence to suggest that this process is related to any modern cellular functions, such as telomere synthesis,[2] though this strategy could, in principle, provide an alternative way to protect eukaryotic chromosome ends. In the present study, only DNA with a tandem repetitive sequence has been synthesized by this method, resembling rolling circle polymerization.[7] From a polymer science perspective, the polymerization process described here is a chain reaction, which is very different from condensation

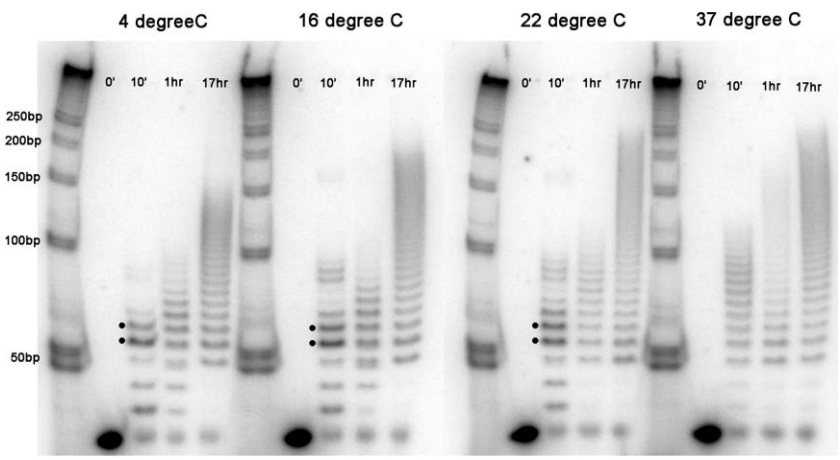

Fig. 3 Temperature effect on the polymerization process. Temperature and polymerization duration are indicated above the gel images.

polymerization.[8] There is more control on chain polymerization than on condensation polymerization. Naturally, we would like to ask what sequence complexity can be achieved by this approach, and whether it is possible to extend this strategy to other biopolymers including proteins and polysaccharides or, even more ambitiously, non-biological synthetic polymers. These questions are important not only for basic study on evolution, but also for many applications including performing DNA computation,[9] modifying DNA nanostructures,[5] and developing biomimetic routes for preparation of polymers with novel structures and functions.

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