Journal of Life Science 2005, Vol. 15. No. 5. 755~762 ©JLS

In vitro Synthesis of Ribonucleic Acids by T7 RNA Polymerase That was Fast Purified with a Modified Procedure

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Received August 5, 2005 / Accepted October 4, 2005

Biochemical amounts of RNA molecules can be synthesized *in vitro*, which is functionally equivalent or similar to those transcripts normally existing at extremely low levels *in vivo*. In this study we described a method for efficient preparation of pure T7 RNA polymerase from *Escherichia coli* strain BL21/pAR1219. The procedure, which used ammonium sulfate fractionation and preparative column chromatography on sephadex SP, was shown to be simple, rapid, and cost effective in comparison with other methods reported previously. Using the purified T7 RNA polymerase we were able to synthesize very long RNA transcript of 1.54 kb length, which is not feasible by conventional chemical synthesis. RNA molecule that was also synthesized by the purified T7 RNA polymerase, such as hammerhead ribozyme, retained its biochemical activity by cleaving the target RNA successfully *in vitro*. Thus, the procedure shown in this study can be useful to synthesize any length of RNA molecules *in vitro* in a simple and cost effective way for a variety of purposes.

Key words – *In vitro* RNA synthesis/ T7 RNA polymerase/ *Escherichia coli*/ Hammerhead ribozyme

Large-scale synthesis of RNAs by T7 RNA polymerase (T7 RNAP) has been applied extensively to the investigation of RNA serving as a biologically active molecule, such as ribozyme[4], transfer RNA[19], and ribosomal RNA[9]. The ability to synthesize RNA in the laboratory is critical to many techniques. Radiolabeled and nonisotopically labeled RNA probes, generated in small scale transcription reactions, can be used in blot hybridizations and nuclease protection assays. Such probes are much more sensitive than random-primed DNA probes. Small scale reactions may also be used to synthesize RNA transcripts containing modified nucleotides for various biochemical and molecular biology studies. Large-scale transcription reactions, generating up to 200 μg of RNA per reaction, can be used for a RNA amplification, expression studies (microinjection, infection with viral transcripts, and *in vitro* translation), structural analysis (protein-RNA binding), and mechanistic studies (ribozyme analyses).

T7 RNAP is a single subunit enzyme with a molecular weight of 98 kDa that is capable of catalyzing transcription without any accessory proteins[17,22]. Other RNA polymerases derived from bacteriophage (SP6 and T3) were also useful for *in vitro* synthesis of RNA. Each RNA polymerase has different DNA promoter sequences for initiation of transcription. The behavior of T7 RNAP is critically dependent on its interaction with the promoter element. The T7 bacteriophage genome contains a variety of promoters that are recognized by T7 RNAP, all of which are related to a 23-base pair consensus sequence (see Fig. 1)[10]. The promoter can be divided into a recognition domain, encompassing positions −17 through −5, and an initiation domain, encompassing positions −4 through +6[5,6,15]. Transcription initiates at the +1 position.

The concentration of commercially available T7 RNA polymerase is lower than that required for most large-scale synthesis projects, and the cost is prohibitively expensive for milligram levels of RNA synthesis. As a consequence, in many laboratories the polymerase is generally isolated from *Escherichia coli* which harbors a plasmid carrying the T7 RNA polymerase gene[8]. Several procedures have been used to purify homogenous and RNase-free T7 RNA polymerase by conventional chromatography[8,12,14,23] or by immobilized metal ion affinity chromatography[2].

The hammerhead ribozyme is the smallest naturally occurring ribozyme identified, which is capable of performing sequence-specific cleavage of RNA[21]. The hammerhead ribozyme is the most widely employed for inhibiting

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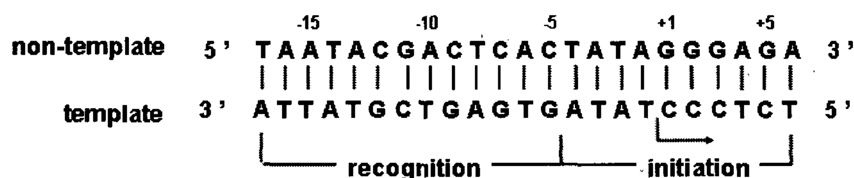

Fig. 1. Consensus T7 RNA polymerase promoter element. The recognition domain encompasses base pairs −17 through −5; the initiation domain encompasses base pairs −4 through +6. Transcription initiates at position +1 (arrow), giving rise to an RNA transcript that is complementary to the template strand.

the function of the target genes[1,3,13,20]. Functional hammerhead ribozyme can be designed to cleave any target RNA *in trans* by generating RNA molecules with sequences reverse complementary to a target RNA in the hybridizing arms that flank a functional ribozyme, core sequence[1,3]. The trans-acting hammerhead ribozyme can cleave any target substrate containing a 5'-NUH-3' triplet (where N represents any nucleotide and H represents A, C, or U)[13,20].

Acute lymphoblastic leukemia (ALL) that is often found in childhood hematopoietic malignancies associated with the chromosomal translocation between chromosomes 12 and 21[11]. The chromosomal translocation t(12;21)(p12;q22) results in fusion of two genes of transcripition factors, TEL and AML1 (TEL-AML1) that encodes a chimeric protein, TEL/AML1 of 93 kDa (see Fig. 4), which is unique to the malignant cell phenotype and is primarily responsible for leukemogenesis[11,18]. In order to test biochemical authenticity of the synthesized RNA by the T7 RNAP, we prepared a hammerhead ribozyme and its RNA substrate of the chimeric mRNA (54 nts of TEL-AML1 RNA containing the fusion junction) by the *in vitro* transcription using the purified T7 RNAP. The hammerhead ribozyme synthesized in this study successfully cleaved the substrate RNA molecule *in vitro*, demonstrating that the T7 RNAP purified in this study was suitable for the synthesis of RNA molecules with diverse purposes. Altogether, we described a simple method for fast purification of T7 RNA polymerase, which can be accomplished in 2 days, and a detailed method of *in vitro* transcription using the polymerase for biochemical synthesis of RNA in a simple and cost effective way.

Materials and Methods

Culture of bacteria

E. coli strain BL21/pAR1219 which carries the gene for T7 RNA polymerase on an ampicillin resistance plasmid[8] was obtained as a gift from Dr. Smita S. Patel (Robert Wood Johnson Medical School, U.S.A.). The polymerase gene is under control of a *lac* promoter and it can be de-repressed by the addition of isopropyl-β-thiogalactoside (IPTG, purchased from Sigma). LB medium (10 g tryptone, 5 g yeast extract, and 10 g NaCl per liter) was used to culture the *E. coli* strain BL21/pAR1219. 100 ml of LB containing 100 μg/ml of ampicillin was incubated with bacteria and grown overnight at 37℃. 1 ml of this starter culture was added to one liter of LB media containing ampicillin. The inoculated medium was incubated for about 2 hr until the absorbance at 600 nm of the culture reached 0.5. IPTG was then added to a final concentration of 0.5 mM to induce the expression of the T7 RNA polymerase. After 4 h, the cells were separated by centrifugation and the pellets were washed twice by 100 ml of wash buffer (20 mM TrisHCl, pH 8.1, 20 mM NaCl, 2 mM EDTA). The washed cells was used immediately or frozen at -70℃ for long-term storage.

Bacterial cell lysis and ammonium sulfate fractionation

The cell pellets were resuspended in 70 ml cold Buffer LB (50 mM TrisHCl, pH 8.1, 20 mM NaCl, 2 mM EDTA, 1 mM DTT). Cell lysis was initiated by adding 17 ml of a fresh solution of egg white lysozyme (1.5 mg/ml in buffer LB), 150 μl of 20 mg/ml Phenylmethylsulfonylfluoride (PMSF, purchased from Sigma), and 70 μl of 5 mg/ml leupeptin purchased from Sigma. The cell lysis mixture containing lysozyme was incubated for 20 min at room temperature. Although the cell line BL21 lacks the major protease that degrades T7 RNAP, the protease inhibitor PMSF was added to prevent any unwanted proteolysis. After incubation 9 ml of 0.8% (w/v) sodium deoxycholate (from Sigma) was slowly added to the cell lysis mixture and it was further incubated for 20 min at room temperature. In order to decrease viscosity of the cell lysate solution, the mixture was sonicated briefly (30 W for 30 sec). To the lysate solution 150 μl of 20 mg/ml PMSF was added and 17 ml of 2 M ammonium sulfate was slowly added with gentle stirring on ice. While

stirring vigorously the solution on ice, 17 ml of 10% (v/v) Polyethyleneimine (PEI, purchased from Sigma) was slowly added. After 20 min of stirring on ice, the cell lysate solution was centrifugated (39,000 g) at 4℃ for 15 min. The resulting supernatant was collected and .slowly mixed on ice with 0.82 volumes of a saturated solution of enzyme grade ammonium sulfate. This solution was kept on ice for 15~20 min with stirring. The solution was precipitated by centrifugation at 12,000 g for 10 min at 4℃. The precipitates were dissolved with 50 ml of 100 mM NaCl plus buffer C (20 mM sodium phosphate, pH 7.7, 1 mM EDTA, 1 mM DTT, 5% glycerol). This fraction was dialyzed overnight against 4 liter of dialysis solution containing buffer C and 100 mM NaCl.

T7 RNAP purification by column chromatography

The dialyzed fraction containing RNA polymerase was centrifuged at 12,000 g for 20 min at 4℃. The supernatant was collected and its conductivity was measured. The supernatant solution was diluted with Buffer C plus 50 mM NaCl solution until its conductivity is equal to that of Buffer C + 50 mM NaCl. The diluted solution was next loaded onto the column (2.5×30 cm) packed with pre-swollen and degassed sephadex SP (C-50) resin obtained from Amersham Biosciences, which was pre-equilibrated with Buffer C plus 50 mM NaCl. Flow rate was adjusted to 0.9 ml/min and 5 ml of each fraction was collected at 4℃. The column was washed with 3 x the volume of the column with Buffer C plus 50 mM NaCl. Column eluant at this stage was collected and analyzed by SDS-PAGE (lane 4 in Fig. 2). The T7 RNAP was eluted with a high slat solution, that is Buffer C plus 200 mM NaCl (lane 5 in Fig. 2). In order to find out the purest fractions of T7 RNAP, each column fractions were analyzed by SDS-PAGE (10%) with molecular weight standards. Fractions containing single protein band of T7 RNAP were collected and pooled for ultrafiltration (YM 30 amicon concentrator). Ultrafiltration under nitrogen gas at 4℃ was performed by adding buffer C plus 50% glycerol and 100 mM NaCl. Concentrated T7 RNA polymerase solution after ultrafiltration was aliquoted for storage at -80℃, and the protein concentration was spectrophotometrically measured using an extinction coefficient of 1.4×10^5 $M^{-1}cm^{-1}$[5]. Activity of the purified T7 RNAP was determined by the previously described method[16]. One unit of T7 RNAP was defined as the amount of 1 nmol of AMP into acid-insoluble material in 1 h at 37℃ during *in vitro* transcription reaction.

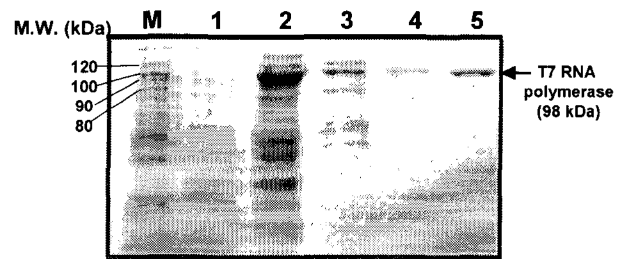

Fig. 2. 10% SDS-PAGE analysis of T7 RNA polymerase after each step of purification. lane M, the protein standards whose molecular weights were 120, 100, 90, 80, 70, 60, 50, 40, 25, and 15 kDa, respectively. Lane 1, the crude extract from uninduced BL21/pAR1219 (no IPTG was added); lane 2, the crude extract from induced BL21/pAR1219; lane 3, the enzyme sample collected after ammonium sulfate fractionation; lane 4 and 5, the T7 RNA polymerase after sephadex SP chromatography elution at low salt (50 mM NaCl) and high salt (200 mM NaCl), respectively. The gel was stained with Coomassie brilliant blue R-250.

In vitro synthesis of RNA and hammerhead ribozyme

The purified T7 RNAP was used for the synthesis of RNA molecule by *in vitro* transcription of linearized plasmid DNA containing T7 promoter. For this purpose a plasmid containing the open reading frame of hepatitis C virus (HCV) NS 3 protein flanked with T7 consensus promoter was digested with restriction enzyme. The linearized plasmid DNA was used as a template for the synthesis of long RNA (1.54 kb) by the purified T7 RNAP. 30 nM of linearized DNA template was mixed with the purified T7 RNAP (25 U/ml) and transcription buffer (15 mM $MgCl_2$, 2 mM spermidine, 5 mM DTT, and 50 mM Tris, pH 7.5) in the presence of 1 mM NTP mixture. The reaction mixture was incubated at 37℃ for 2 h. To remove the DNA template DNase (RNase-free, 0.25 U/μl) was added after the incubation and the reaction was further incubated at 37℃ for 30 min. The entire reaction (100 μl) was quenched by adding EDTA (final concentration of 15 mM) to the reaction mixture. The quenched reaction mixture was mixed with the same volume of gel loading buffer (8 M urea, 0.08% bromophenol blue, 10% glycerol) and heated at 90℃ for 1 min. The synthesized RNAs were analyzed by the denaturing-PAGE (4%) containing 8 M urea. The RNA bands were identified by the UV-shadowing method and were excised from the gel for purification of the *in vitro* synthesized RNA.

Hammerhead ribozyme (see Fig. 4) used in this study was prepared by *in vitro* transcription. DNA template for hammerhead ribozyme, 5'-TTG GGA GAA TTT CGT CCT

CAC GGA CTC ATC AGG CAG AAT GCC TAT AGT GAG TCG TAT TAG TCC-3′, which was synthesized chemically and purchased from Bioneer (Daejeon, Korea), contained template sequence of the T7 promoter (underlined sequence, refer to Fig. 1). The transcription mixture contained 0.4 μM DNA template, 0.8 μM annealing oligodeoxynucleotide primer having the sequence 5′-GGA CTA ATA CGA CTC ACT ATA-3′ (T7 promoter non-template sequence underlined), 2 mM each of the four NTPs, and 25 units/ml T7 RNA polymerase. The transcription product was purified by denaturing PAGE and eluted from the gel as stated above. The DNA template for TEL-AML1 chimeric RNA consisted of the sequence from 16 nt 5′ of the TEL-AML1 junction to 38 nt 3′ of the TEL-AML1 junction (Fig. 4). T7 transcription *in vitro* and gel-electrophoretic purification of the TEL-AML1 mRNA substrates was performed as described above.

In vitro cleavage of RNA with hammerhead ribozyme

Target RNA cleavage activity of ribozyme was tested in 20 mM $MgCl_2$ and 50 mM Tris (pH 7.5) under single-turnover (enzyme saturating) conditions with incubation at 37℃. Tri-phosphate groups from the 5′ end of the substrates RNA were removed with calf intestinal alkaline phosphatase (1 unit/ml, New England Biolabs) and the dephosphorylated transcripts were then labeled using T4 polynucleotide kinase (purchased from USB) and [γ-^{32}P]ATP. 5′-end labeled substrate RNAs were purified by denaturing Urea-PAGE. For the test of RNA cleavage activity of ribozymes, a trace amount of ^{32}P-5′-labeled substrate RNA was mixed with unlabeled substrate (to yield a final concentration of 25 nM RNA) and ribozyme (50 nM final concentration), and incubation was performed using the condition as above. The reactions were quenched at various time points by adding an equal volume of the gel-loading buffer containing 25 mM Na_2EDTA and 18 M urea. The products were analyzed by 8 M urea-containing denaturing PAGE (8%) and detected by autoradiography.

Results and Discussion

Purification of T7 RNA polymerase

We purified the T7 RNA polymerase that was expressed in *E. coli* with a method of ammonium sulfate fractionation and ion-exchange column chromatography. The procedure in the present work is a rapid, simple, and inexpensive method for purification of RNase-free T7 RNA polymerase for synthesis of large amounts of RNA *in vitro*. The whole procedure of purification can be completed in 2 days with a yield of about 20 mg T7 RNA polymerase started from 660 mg of total protein obtained from 2 liter culture of *E. coli*. T7 polymerase was fractionated to a purity over 95% as judged by lack of extra bands on SDS-PAGE (lane 5 in Fig. 2) after ammonium sulfate fractionation and subsequent sephadex SP column chromatography. The whole procedure of purification and its result was summarized in Table 1. Polymerase purified by our procedure has a specific activity of about 720,000 units/mg which is close to that reported previously[19]. One unit of T7 RNA polymerase is defined as the amount of enzyme required to catalyze the incorporation of 1 nmol of AMP into acid-insoluble material in1 h at 37℃ using a plasmid containing a T7 promoter as template[16]. The enzyme was very stable when stored at -20℃ with a high concentration (2 mg/ml) for at least 1 year.

The purification procedure differs in several aspects from previously described methods[8,12,14,23]. First, Polymin P and/or ammonium sulfate precipitation employed in other methods[8,12,14,23] was modified to a combined methods of cell lysis with lysozyme and subsequent ammonium sulfate fractionation. Second, the chromatographic procedure was decreased from two or three columns to one. The method developed by Wyatt *et al.*[23] and Li *et al.*[16] used two-step chromatography for the purification. However, it did not yield homogenous polymerase and RNase activity had to be monitored during chromatography[23]. In contrast, the procedure shown in this paper provides a much simpler method to obtain T7 RNAP using a single step of column chromatography. Of importance, the sephadex SP resin

Table 1. Purification of the T7 RNA Polymerase from *E.coli* BL21/pAR1219

Step	Total protein (mg)	Volume (ml)	Total activity (10^5 unit)	Specific activity (10^5 unit/mg)	Yield (%)	Purification fold
Crude extract*	660	100	480	0.73	100	1.0
Ammonium sulfate fractionation	130	50	340	2.6	71	3.6
Sephadex SP chromatography	20	10	144	7.2	30	9.9

*The crude extract shown here is from about 10 g of cell paste following sonication and centrifugation.

containing a sulphopropyl group, which is working as a cation exchanger, separated T7 RNAP very efficiently from other proteins. Finally, this procedure showed good reproductivity so that it was not necessary to probe for RNases during purification. Usually from 10 g of wet cells we could get 20 mg of enzyme, which was enough for preparing a 500 ml transcription reaction.

RNA synthesis *in vitro* by the purified T7 RNA polymerase

The activity of the purified T7 RNAP was evaluated with a capability of synthesizing RNA *in vitro*. The purified T7 RNAP synthesized RNA transcripts with an amount of hundreds of microgram per reaction using the DNA template at 37℃ for 2 h. Fig. 3A shows synthesized RNA molecules with an expected size of 1.54 kb. Main band appeared under short wavelength of UV light with some smearing pattern. These smearing pattern of the RNA bands are likely to be resulted from either degradation or premature termination of the transcription. Since RNA molecules are prone to be degraded due to their unstable chemical structure, they have to be handled very carefully. Thus, maintaining the RNase-free condition was crucial for the success of *in vitro* transcription. We have isolated the synthesized RNA molecules from the urea-gel and extracted the RNA band. The purified RNA was reloaded onto the denaturing urea-PAGE with a molecular weight markers to identify that the RNA of desired length was synthesized (Fig. 3B). The urea-PAGE showed a clear RNA band with a molecular weight of 1.54 kb. Therefore, the purified T7 RNA polymerase was useful to prepare moderate amount of RNA molecules with a length longer than 1 kb. Moreover, urea-PAGE purification of the RNA transcripts provided RNA molecules with a homogeneous length.

The yield of RNA synthesized *in vitro* was known to be improved by adding the inorganic pyrophosphatase, which cleaves pyrophosphate into each inorganic phosphate ($PP_i \rightarrow 2P_i$), in the *in vitro* RNA synthesis reaction[7]. Pyrophosphate is a byproduct of RNA synthesis, which was resulted from NTP incorporation into the growing RNA chain, and impedes the efficiency of RNA synthesis. We have included the inorganic pyrophosphate in the RNA synthesis reaction mixture to a final concentration of 0.02 U/μl. Compared with the reaction without the inorganic pyrophosphtase, turbidity of the reaction mixture containing the enzyme was decreased after 2 hr of RNA synthesis reaction (data not

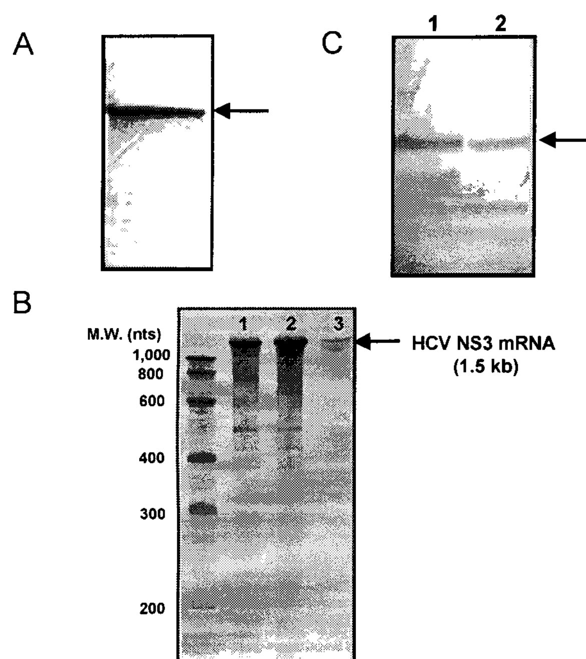

Fig. 3. Denaturing urea-PAGE (4%) analysis of RNA synthesized *in vitro*. (A) HCV NS3 RNA (1.54 kb, arrow) synthesized *in vitro* using the purified T7 RNA polymerase. The image was taken under UV-shadowing. (B) RNA bands shown in panel (A) was excised and purified. lane 1 and 2; before the gel-purification (two different batches), lane 3; HCV NS3 RNA transcript after gel-purification. (C) Pyrophosphatase enhances the yield of RNA synthesis. Arrow indicates the RNA synthesized *in vitro* by T7 RNAP. lane 1; RNA synthesized in the presence of inorganic pyrophosphatase, lane 2; RNA synthesized in the absence of inorganic pyrophosphatase, The same volume of reaction was loaded in each lane.

shown). Since the inorganic pyrophosphatase cleared up pyrophosphate that can be polymerized to polyphophosphate during the RNA synthesis reaction, the reaction mixture was not turbid after the reaction. When the RNAs synthesized *in vitro* by T7 RNAP with or without inorganic pyrophosphatase were analyzed with denaturing Urea-PAGE, the yield of synthesized RNA was improved by about two-fold in the presence of the inorganic pyrophosphatase in the reaction (Fig. 3C). Thus, removal of pyrophosphate enhances the capability of T7 RNA polymerase to synthesize RNAs *in vitro* by driving an equilibrium to the synthesis of RNA.

Biochemical test of synthesized RNA: RNA cleavage with hammerhead ribozyme

We next performed to synthesize short RNA molecule with DNA template containing T7 promoter sequence. DNA

template was annealed with oligo DNA of sense sequence of T7 promoter. Thus, the purified T7 RNAP was used to *in vitro* RNA synthesis with the annealed DNA template containing T7 promoter sequence shown in Fig. 1. In addition, to address whether the synthesized RNA molecule was biochemically authentic, we have prepared the hammerhead ribozyme composed of 41 nts and investigated cleavage activity against the target substrate RNA. As depicted in Fig. 4B, The ribozyme was designed to recognize one of its target sites and cleave TEL/AML1 chimeric RNA that is causative of acute lymphoblastic leukemia (ALL). Ribozyme substrate, part of the TEL/AML1 chimeric RNA (54 nts of oligo RNA containing junction sequence, see Fig. 4A) was also synthesized *in vitro* by the purified T7 RNAP. The purified T7 RNAP synthesized both the ribozyme and the RNA substrate successfully, which were used for subsequent RNA cleavage assay. As shown in Fig. 5, the hammerhead ribozyme prepared by T7 RNAP was capable of cleaving the target RNA substrate in a time-dependent manner. The cleavage product was appeared at the expected size of 14 nts. These results clearly demonstrate that the T7

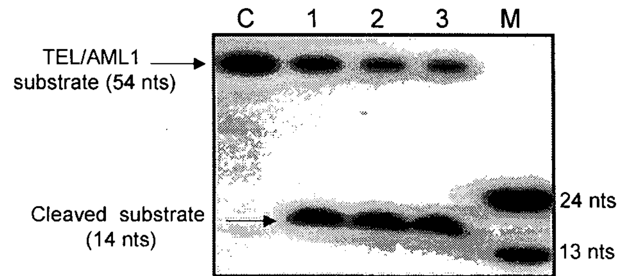

Fig. 5. *In vitro* cleavage analysis of TEL/AML1 targeted hammerhead ribozyme. ^{32}P-labeled TEL/AML1 RNA substrate (25 nM) and the hammerhead ribozyme (50 nM) that were both synthesized by the T7 RNAP were incubated for different time at 37℃ in the presence of 20 mM $MgCl_2$. Following cleavage reaction, the products were separated by denaturing PAGE (10%) and the gel was subjected to autoradiography. Lane 1, 2, and 3 represents the cleavage reaction for 30 min, 1 hr, and 2 hr, respectively, and lane C shows a reaction without the ribozyme. Lane M shows a molecular weight marker that was 5′-end labeled oligo DNA.

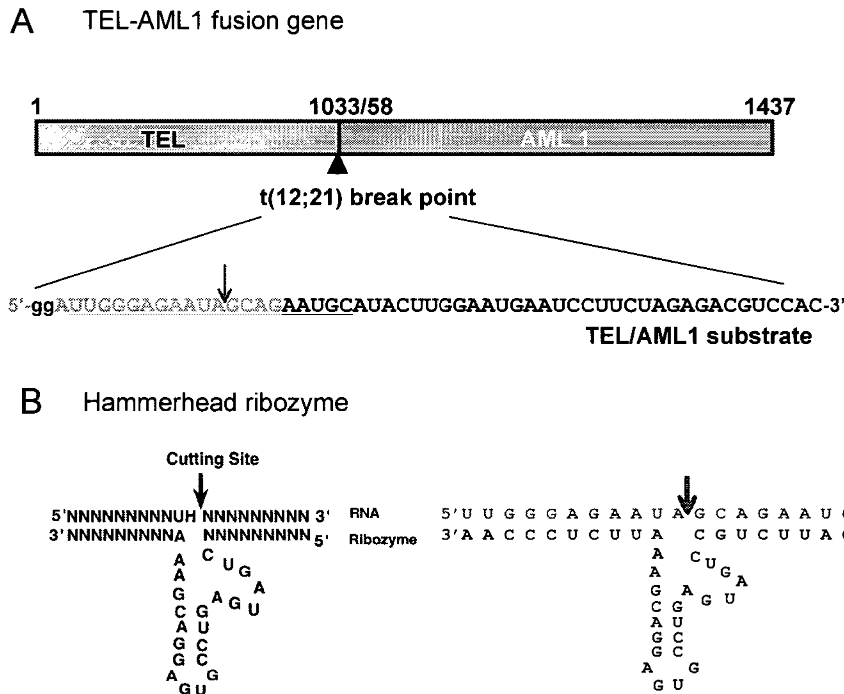

Fig. 4. TEL-AML1 fusion mRNA and hammerhead ribozyme. (A) TEL-AML1 fusion gene that is resulted from chromosomal translocation is depicted (grey-colored bases and black-colored bases represent sequences of TEL and AML1, respectively). Part of the chimeric RNA of 54 nts was used for substrate of the hammerhead ribozyme. Underlined sequence represents the portion that is recognized by the ribozyme and arrow indicates the expected cleavage site. Two G (shown in lower case) were introduced at the 5′ end of the TEL/AML1 substrate RNA sequence for efficient transcription by the T7 RNAP. (B) Hammerhead ribozyme recognizes the target site and cleave after any 5′-NUH-3′ sequence (see text for detail). Right panel shows the hammerhead ribozyme designed in this study, which targets the TEL/AML1 chimeric RNA.

RNAP can be used to prepare ribozyme molecules with desired sequence and activity.

In the present study we introduced a simple method for fast purification of T7 RNA polymerase, which can be accomplished in 2 days using a single column chromatography. Polymerase purified by this procedure was demonstrated to be useful to synthesize RNA *in vitro* with a various lengths ranging from 50 to more than 1,000 bases long. Hence, these procedures will be beneficial for *in vitro* transcription to prepare biochemical amount of RNA and ribozymes in a simple and cost effective way.

Acknowledgment

Authors thank professor Sung Key Jang (Department of Life Science, POSTECH, Korea) for a kind gift of plasmid containing HCV NS3 open reading frame. This work was supported by Korea Research Foundation Grant (KRF-2004-015-C00327).

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초록 : 변형된 방법으로 신속히 정제된 T7 RNA 중합효소를 이용한 리보핵산의 시험관 내 합성

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세포 내에서 매우 적은 양으로 존재하는 RNA 전사체와 기능적으로 동일하거나 비슷한 RNA를 RNA 중합효소를 써서 *in vitro*에서 생화학적으로 의미 있는 양 만큼을 합성할 수 있다. T7 RNA 중합효소를 발현하는 재조합 유전자를 지닌 대장균주 BL21/pAR1219로부터 순수한 T7 RNA 중합효소를 손쉽게 얻는 방법을 본 논문에서 소개한다. 황산암모늄 분획화와 sephadex SP 컬럼 크로마토그래피법으로써 여타의 방법과 비교하여 더 간단하고 빠르게, 그리고 경제적으로 T7 RNA 중합효소를 분리할 수 있었다. 정제된 T7 RNA 중합효소를 이용하여 보통의 화학적 합성법으로 불가능한 긴 길이(1.54 kb)의 RNA 전사체를 합성 하였다. 한편, 정제된 T7 RNA 중합효소에 의해 생성된 망치머리 리보자임은 표적 RNA를 *in vitro*에서 절단함으로써, 생성된 RNA가 생화학적 기능성을 유지한다는 것을 입증하였다. 따라서 본 연구에서 소개되는 절차들은 다양한 길이의 RNA를 목적에 따라 간단하고 경제적으로 합성하는데 유용하게 이용될 수 있다.