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Non-enzymatic, template-directed ligation of 2′-5′ oligoribonucleotides. Joining of a template and a ligator strand

Hiroaki Sawai*, Shuichi Totsuka, Kenji Yamamoto and Hiroaki Ozaki

Department of Chemistry, Faculty of Engineering, Gunma University, Kiryu, Gunma 376-8515, Japan

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ABSTRACT

Decauridylate containing exclusively a 2′-5′ phosphodiester bond ([2′-5′]U_{10}) served as a template for the synthesis of oligoadenylates [oligo(A)s] from the 5′-phosphorimidazolide of 2′-5′ diadenylate (ImpA-2′p5′A). Joining of [2′-5′]U_{10} and ImpA2′p5′A also took place in substantial amounts to yield long-chain oligoribonucleotides in the template-directed reaction. An unusual CD spectrum ascribed to helix formation between [2′-5′]U_{10} and [2′-5′](pA)$_2$ was observed under the same conditions as that of the template-directed reaction. The 3′-5′ linked decauridylate ([3′-5′]U_{10}) also promoted the template-directed synthesis of oligo(A)s from ImpA2′p5′A, but more slowly compared with [2′-5′]U_{10}. The results indicate that short-chain RNA oligomers with a 2′-5′ phosphodiester bond could lead to longer oligoribonucleotides by template-directed chain elongation.

INTRODUCTION

It has been proposed that RNA could have played the roles of information carrier and catalyst in the prebiotic era (1,2). Contemporary RNA exclusively has a 3′-5′ phosphodiester bond. However, 2′-5′ linked oligoribonucleotides are chemically feasible and are formed in model processes of prebiotic synthesis of RNA. For example, 2′-5′ linked oligoribonucleotides are mainly formed from activated mononucleotides in the presence of a divalent metal (3,4) or uranyl ion catalyst (5,6). Template-directed oligomerization of the activated nucleotides also produces mainly 2′-5′ linked oligoribonucleotides in the non-enzymatic model reaction of RNA replication (7). On the other hand, 3′-5′ linked oligoribonucleotides are preferentially formed in oligomerization by a clay mineral catalyst (8–10) or in template-directed oligomerization of 2-methylimidazole-activated guanylate on a 3′-5′-linked poly(C) template (11–14). The Zn^{2+}-catalyzed oligomerization of guanosine 5′-phosphorimidazolide on 3′-5′-linked poly(C) also yields 3′-5′-linked oligoguanylates [oligo(G)s], while the Pb^{2+} catalyst yields mainly 2′-5′-linked oligo(G)s under the same conditions (15). Lohrmann and Orgel reported that the 2′-OH group of ribonucleotide is more reactive than the 3′-OH group and the 2′-5′ phosphodiester bond is formed more easily than the 3′-5′ bond in non-enzymatic oligoribonucleotide synthesis (16), although the reactivity of each OH group is affected by the ribose ring conformation and by a steric effect imposed by the activated group or a template. Thus 2′-5′- and 3′-5′-linked oligoribonucleotides could have been formed during chemical evolution and possibly served as a template for complementary oligoribonucleotide synthesis from mono- or short-chain oligoribonucleotides. The 3′-5′-linked poly- or oligoribonucleotides have been proved to serve as a template in the non-enzymatic oligomerization or ligation of oligoribonucleotides (7,15–17). Very recently, Ferris *et al*. reported the template-directed synthesis of oligo(G)s from activated guanylate on 2′-5′-linked oligo(C)s formed by a clay mineral catalyst (18,19). Prakash *et al*. have also demonstrated 2′-5′-linked oligo(C)s in a hairpin template-promoted oligomerization of the 2-methylimidazolide of guanosine-5′-phosphate (2-MeImpG) in the presence of Mg^{2+}. They have shown that the 2′-5′-linked oligo(C) template is less efficient than the 3′-5′-linked oligo(C) for oligomerization of 2-MeImpG (20). However, template-directed ligation of 2′-5′-linked short-chain oligoribonucleotides on the complementary 2′-5′- or 3′-5′-linked oligoribonucleotides, which could take place in the prebiotic chemical process, has been a matter of speculation.

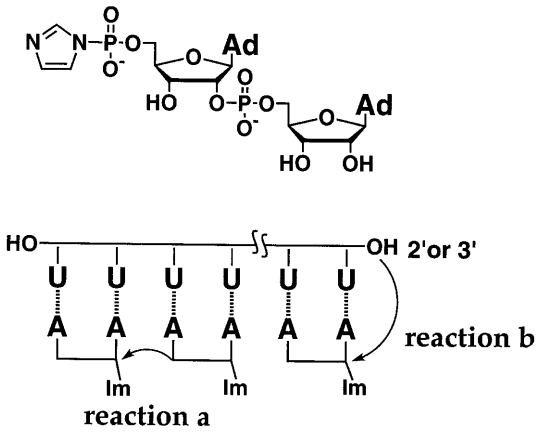
In this study, we investigated helix formation and non-enzymatic template-directed ligation of 2′-5′-linked diadenylate on 2′-5′- or 3′-5′-linked decauridylate, [2′-5′]U_{10} or [3′-5′]U_{10}. The 2′-5′-linked oligonucleotides were formed from the activated nucleotide by a uranyl ion catalyst. We found that the [2′-5′]U_{10} template promoted ligation of the 2′-5′ diadenylate to yield oligo(A)s faster than [3′-5′]U_{10} (reaction a, Scheme 1). Moreover, the 2′-5′ diadenylate substantially condensed to the template [2′-5′]U_{10}, as shown in Scheme 1, reaction b.

MATERIALS AND METHODS

Materials

Poly(A), poly(U), adenosine 5′-monophosphate, uridine 5′-monophosphate and nuclease P1 (NP1) were from Yamasa (Tokyo). Snake venom phosphodiesterase (SVPD) and bacterial alkaline phosphatase (BAP) were from Worthington Biochemical (Freehold, NJ). Nuclease SW was a generous gift from

*To whom correspondence should be addressed. Tel: +81 277 30 1220; Fax: +81 277 30 1224; Email: sawai@gunma-u.ac.jp



Scheme 1. ImpA2′p5′A and template-directed ligation on the [2′-5′]U_{10} template.

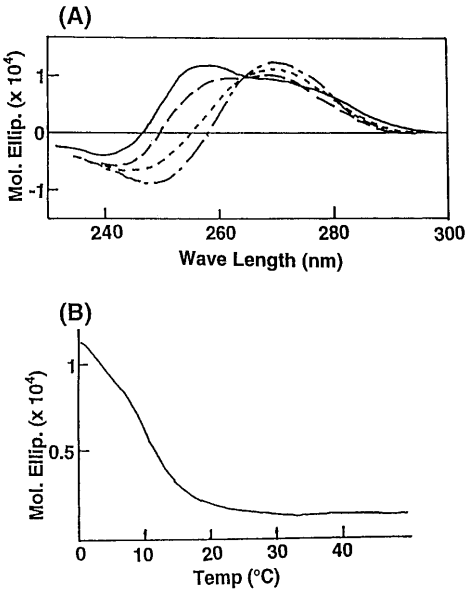


Figure 1. CD spectra of the helix composed of [2′-5′](pA)$_2$ and [2′-5′]U_{10}. (**A**) The measurement was carried out at 10 mM [2′-5′](pA)$_2$ and 20 mM [2′-5′]U_{10} concentrations in the presence of 30 mM $MgCl_2$ and 0.2 M NaCl in 0.2 M *N*-ethylmorpholine buffer (pH 7.0) using a 0.0025 cm path length cuvette at 0 (——), 5 (– – –), 10 (· · ·) and 30°C (– · –).(**B**) Melting curve constructed from CD at 260 nm.

Dr J.Mukai (Kyushu University, Japan). All other materials were obtained commercially. The 2′-5′-linked oligo(A)s ([2′-5′](pA)$_n$, n = 2, 4 and 6) were prepared by UO_2^{2+} ion-catalyzed polymerization of adenosine 5′-phosphorimidazolide (ImpA) in aqueous solution by the published procedure (5,21). The phosphorimidazolide of 2′-5′-linked diadenylate (ImpA2′p5′A) was prepared from [2′-5′](pA)$_2$ and imidazole by the same procedure as for the synthesis of ImpA (5,16).

Synthesis of templates [2′-5′]U_{10} and [3′-5′]U_{10}

The 2′-5′-linked decauridylate ([2′-5′]U_{10}) was prepared by UO_2^{2+}-catalyzed polymerization of uridine 5′-phosphorimidazolide (ImpU) by the literature procedure (5,21), followed by treatment with NP1 and BAP to concomitantly degrade 3′-5′ linkages and the 5′-terminal phosphate. The 2′-5′-linked oligo(U)s were purified by DEAE–Sephadex ion exchange column chromatography using linear gradient elution with triethylammonium hydrogen carbonate buffer (0–1.0 M). Fractions of [2′-5′]U_{10} were collected and dialyzed several times to remove triethylammonium hydrogen carbonate completely. The 3′-5′-linked decauridylate ([3′-5′]U_{10}) was prepared by partial digestion of poly(U) with nuclease SW (21) followed by treatment with BAP to cleave the 5′-terminal phosphate and purified by the same procedure as described for [2′-5′]U_{10}. The purity of the oligoribonucleotides was determined by HPLC.

HPLC

HPLC analyses were performed on an RPC-5 column with linear gradient elution with $NaClO_4$ (0–0.05 M) buffered with 2.5 mM Tris–acetate (pH 7.5) and 0.1 mM EDTA or an ODS-Silicagel column with a linear gradient of acetonitrile (2–30 %) buffered with 0.1 M triethylammonium acetate (pH 7.0).

UV spectroscopy and oligoribonucleotide concentration

UV spectra were taken with a Hitachi 3200 spectrophotometer. Oligoribonucleotide concentrations were determined by UV absorbance at 260 nm using the residual molar extinction coefficients of A and U, $\varepsilon_{260} = 15.3 \times 10^3$ and 10.0×10^3/M/cm respectively, after correcting for the hypochromicity of each oligoribonucleotide. Hypochromicity of the oligoribonucleotides was determined by the ratio of absorbance at 260 nm in Tris buffer (pH 7.0) before and after alkaline hydrolysis of the oligoribonucleotide. The hypochromicity value was the mean of five determinations. The hypochromicities of [2′-5′](pA)$_2$, [2′-5′](pA)$_4$, [2′-5′](pA)$_6$, [2′-5′]U_{10} and [3′-5′]U_{10} were 18, 25, 28, 15 and 10% respectively. All oligomer concentrations are expressed based on a monomer equivalent.

Circular dichroism (CD) spectroscopy and melting studies

CD spectra were recorded with a JASCO-720 spectropolarimeter at several temperatures with a PTC-343 temperature controller. The CD results were expressed in terms of [θ] (molecular ellipticity). CD versus temperature plots were recorded at 260 or 265 nm measuring at every 0.1°C with a temperature increase rate of 0.25°C/min. The melting temperature was determined from the first differential of the CD melting curve. The CD measurements of the combinations [2′-5′](pA)$_2$:[2′-5′]U_{10} and [2′-5′](pA)$_2$:[3′-5′]U_{10} were carried out at high oligoribonucleotide concentrations using a 0.0025 cm path length cuvette to obtain information on helix formation under the conditions of template-directed synthesis.

Template-directed condensation

A reaction mixture (50 µl) containing 0.01 M ImpA2′p5′A, 0.02 M [2′-5′]U_{10} or [3′-5′]U_{10}, 0.03 M $MgCl_2$, 0.2 M NaCl and 0.2 M *N*-ethylmorpholine buffer (pH 7.0) was prepared at 0°C and allowed to stand for 28 days at 0°C. Aliquots of the mixture were

taken out after 1, 3, 7, 10, 14 and 21 days and analyzed by HPLC. A control reaction of ImpA2′p5′A without template was also done under the same conditions as for the template-directed condensation.

Characterization of the reaction products

Identification of the reaction products, [2′-5′](pA)$_4$, [2′-5′](pA)$_6$, [2′-5′]A$_2$, [2′-5′]A$_4$ and pA2′pA3′pA2′pA, was carried out by comparison of HPLC retention times with those of authentic samples. Characterization of other minor oligo(A)s containing a partial 3′-5′ linkage was not attempted. However, digestion of the reaction mixture with NP1 was conducted to degrade only the 3′-5′ linkage, leaving only 2′-5′ oligoribonucleotides, which were analyzed by HPLC. Long-chain products, which were formed by condensation between ImpA2′p5′A and template [2′-5′]U$_{10}$, were isolated by preparative HPLC on ODS-Silicagel. The isolated oligoribonucleotides were characterized by two different enzyme digestion systems. Degradation of the oligoribonucleotide with SVPD and BAP was carried out to analyze the ratio of adenosine to uridine in the oligoribonucleotide. SVPD cleaves both the 2′-5′ and 3′-5′ phosphodiester bonds of the oligonucleotide to yield the component 5′-mononucleotides, whose 5′-phosphates were cleaved by BAP. Enzyme digestion was carried out in a mixture (50 µl) containing 0.5 U SVPD, 0.2 U BAP, 0.1 OD$_{260}$ substrate oligoribonucleotide and 0.01 M MgCl$_2$ in 0.02 M Tris–HCl buffer (pH 8.0) at 37°C for 8 h. Treatment of the oligoribonucleotides with NP1 was further conducted to analyze the type of linkage formed in the template-directed reaction, because NP1 degrades only the 3′-5′ linkage. The NP1 digestion was carried out in a mixture (50 µl) containing 10 µg NP1 and 0.25 OD$_{260}$ substrate oligoribonucleotide in 0.01 M ammonium acetate buffer (pH 5.3) for 8 h at 37°C. The molecular weight of the isolated long-chain oligoribonucleotides was further analyzed in a PE-Sciex API-100 electrospray mass spectrometer.

RESULTS AND DISCUSSION

CD of helices composed of [2′-5′](pA)$_2$ and [2′-5′]U$_{10}$ or [3′-5′] U$_{10}$

We have examined helix formation between [2′-5′](pA)$_2$ and [2′-5′]U$_{10}$ or [3′-5′]U$_{10}$ by CD under the conditions of template-directed condensation of ImpA2′p5′A on U$_{10}$ template using a 0.0025 cm cuvette. Figure 1A shows the CD spectrum of the mixture of 0.01 M [2′-5′](pA)$_2$ and 0.02 M [2′-5′]U$_{10}$ in the presence of 0.03 M Mg^{2+} at 0, 5, 10 and 30°C. The spectrum at 0°C, shown by a solid line, has a peak at 255 nm which is ascribed to the triple helix formed from 2′-5′-linked oligo(A) and 2′-5′-linked oligo(U) in the presence of Mg^{2+} ion (22). This unique CD spectrum suggests that the triplex composed of the 2′-5′-linked oligo(A) and oligo(U) has a different conformational form from that composed of 3′-5′-linked RNAs. The peak at 255 nm disappeared with rising temperature and a CD band with a new peak at 270 nm appeared, which is ascribed to a mixture of the single-stranded [2′-5′](pA)$_2$ and [2′-5′]U$_{10}$. The CD spectrum of the mixture at 30°C is essentially the same as that of the sum of the component strands under the same conditions at 30°C (data not shown). Figure 1B shows the CD melting curve of the triplex composed of [2′-5′](pA)$_2$ and [2′-5′]U$_{10}$, which was monitored by CD at 260 nm. The CD amplitude versus temperature profile indicates that helix formation takes place below 20°C. Analysis

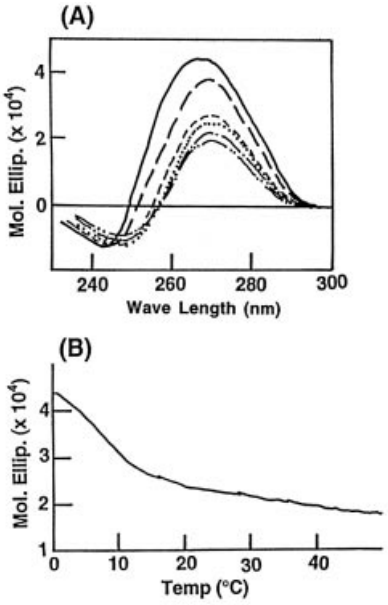


Figure 2. CD spectra of the helix composed of [2′-5′](pA)$_2$ and [3′-5′]U$_{10}$. (**A**) The measurement was carried out at 10 mM [2′-5′](pA)$_2$ and 20 mM [3′-5′]U$_{10}$ concentrations in the presence of 30 mM MgCl$_2$ and 0.2 M NaCl in 0.2 M *N*-ethylmorpholine buffer (pH 7.0) using a 0.0025 cm path length cuvette at 0 (——), 5 (– – –), 15 (- - -) and 20 (· · ·), 35 (–·–) and 50 °C (–· ·–). (**B**) Melting curve constructed from CD at 270 nm.

of the first differential curve allows determination of the melting temperature of the helix as 8.5°C. Figure 2A shows the CD spectrum of a 1:2 mixture of [2′-5′](pA)$_2$ and [3′-5′]U$_{10}$ under the same conditions as for the mixture of [2′-5′](pA)$_2$ and [2′-5′]U$_{10}$. Triple helix formation between [2′-5′](pA)$_2$ and [3′-5′]U$_{10}$ was observed as an increased CD band and shorter wavelength shift of the peak CD around 270 nm (22); the CD band decreased considerably at high temperature, reflecting melting of the helix to the single-stranded components. The melting curve of the helix monitored by CD at 270 nm is shown in Figure 2B. The melting temperature, which was estimated from the first differential of the melting curve, was 5.9°C. The CD studies of helix formation indicate the possibility of template-directed condensation of 2′-5′ diadenylate on the [2′-5′]U$_{10}$ or [3′-5′]U$_{10}$ template at 0°C.

Template-directed ligation

Figure 3A illustrates the HPLC profiles of the reaction products from ImpA2′p5′A in the presence of the [2′-5′]U$_{10}$ template at 0°C. After 28 days the starting ImpA2′p5′A disappeared and the condensation products, tetra- and hexaadenylates, were formed in 21 and 1.5% yields respectively, in addition to large amounts of two long-chain ligation products from [2′-5′]U$_{10}$ and pA2′p5′A, **I** and **II** (Table 1). The main peak in the fraction of tetraadenylate was fully 2′-5′-linked (pA)$_4$ and the subsidiary peak after the main peak was tetraadenylate with an internal 3′-5′ linkage, pA2′pA3′pA2′pA. The molar ratio of the resulting linkage isomers of the tetraadenylate, pA2′pA2′pA2′pA to pA2′pA3′pA2′pA, was 81:19%. The template-directed synthesis reaction mixture was further subjected to NP1 digestion. NP1 degrades only the 3′-5′ linkage, to yield fully 2′-5′-linked

oligonucleotides or a mononucleotide with a 5′-terminal phosphate, although concomitant phosphatase digestion gave a small amount of 5′-dephosphorylated products. The results of the NP1 digestion demonstrates that fully 2′-5′-linked oligonucleotides were the main products of template-directed synthesis. With a [3′-5′]U_{10} template instead of [2′-5′]U_{10}, a small amount of the starting ImpA2′p5′A survived even after 28 days, and tetra- and hexaadenylates were formed in 8 and 0.5% yields respectively (Fig. 3B). The internal linkage of the tetraadenylate formed in this reaction was mainly the 2′-5′ phosphodiester bond, as shown in Table 1. No long-chain oligoribonucleotide corresponding to compound **I** or **II** was obtained from ImpA2′p5′A with [3′-5′]U_{10}. Tetraadenylate was formed in very small amounts and the starting ImpA2′p5′A survived in substantial amounts in the control reaction without template (Fig. 3C). The half-life of hydrolysis of the phosphorimidazolide bond of ImpA2′p5′A was 17 days at 0°C and pH 7.0 in the absence of template. Figure 4 exhibits the time course of the condensation reaction of ImpA2′p5′A on [2′-5′] or [3′-5′]U_{10} template. The reaction was almost complete in 14 days with a [2′-5′]U_{10} template but required over 30 days with a [3′-5′]U_{10} template. The [2′-5′]U_{10} template promoted condensation of ImpA2′p5′A more efficiently than the [3′-5′]U_{10} template, although helix formation was observed in both cases at 0°C under the same conditions.

Characterization of the reaction products

Identification of the ligation products was carried out by comparison of the HPLC with that of an authentic sample or selective enzyme digestion. Structure analysis of the long-chain ligation products **I** and **II** was performed on fractions isolated by reverse phased ODS-Silicagel HPLC. We used ODS-Silicagel HPLC for preparative purposes, because the sample charging capacity of the RPC-5 column is too small to obtain sufficient amounts of the samples for characterization. Enzyme digestion of compounds **I** and **II** with SVPD and BAP yielded adenosine and uridine in ratios of nearly 10:2 and 10:4, respectively. The result indicates that compound **I** was formed by condensation of [2′-5′]U_{10} and ImpA2′p5′A and **II** from **I** and another ImpA2′p5′A. The structure of compounds **I** and **II** was also confirmed by their molecular weight determination by electrospray mass spectrometry. The observed masses of **I** and **II** were 3762.2 and 4425.6 respectively, which correspond to the calculated masses of $U_{10}A_2$-triethylammonium (mol wt 3762.2) and $U_{10}A_4$-triethylammonium (mol wt 4424.6), respectively. Compounds **I** and **II** were further treated with NP1 to determine the type of phosphodiester bond between [2′-5′]U_{10} and [2′-5′]A_2. Substantial amounts of 2′-5′U_{10} and pA2′p5′A were formed in addition to the intact form by NP1 digestion of **I**. In compound **I**, [2′-5′]U_{10}2′pA2′pA was resistant to NP1, while [2′-5′]U_{10}3′pA2′pA was cleaved to [2′-5′]U_{10} and pA2′p5′A. NP1 digestion of compound **II** yielded fully 2′-5′-linked $U_{10}A_2$, U_{10}, A_4 and A_2, in addition to intact $U_{10}A_4$. The result indicates that compound **II** is a mixture of [2′-5′]U_{10}3′pA2′pA2′pA2′pA, [2′-5′]U_{10}3′pA2′pA3′pA2′pA, [2′-5′]U_{10}2′pA2′pA2′pA2′pA and [2′-5′]U_{10}2′pA2′pA3′pA2′pA. The yields and the types of linkage of **I** and **II** formed from ImpA2′p5′A and template [2′-5′]U_{10} are listed in Table 1. [2′-5′]U_{10} and pA2′pA were joined by 2′-5′ and 3′-5′ phosphodiester bonds in nearly the same ratio. The possibility of a 5′-5′ phosphodiester bond between [2′-5′]U_{10} and pA2′pA could be excluded by the fact that only the

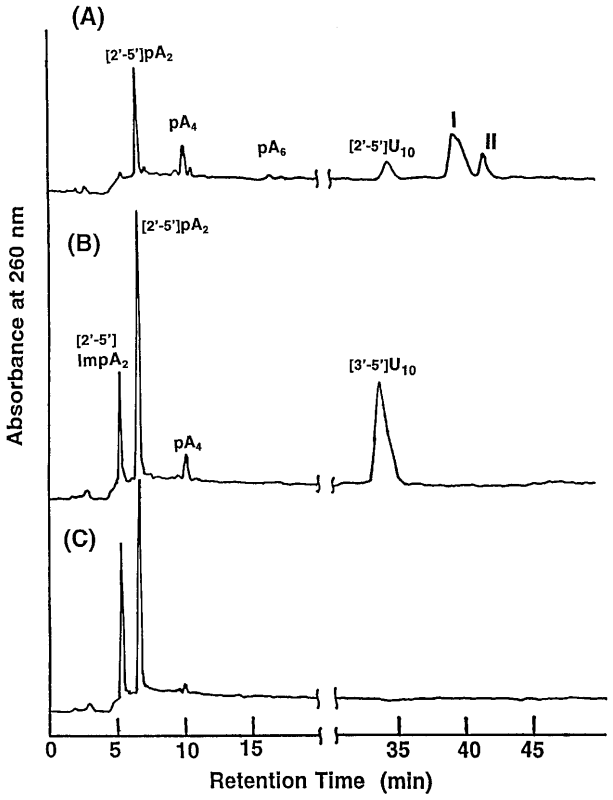


Figure 3. HPLC profile of the reaction products from template-directed condensation of ImpA2′p5′A. (**A**) Reaction on [2′-5′]U_{10} template. Template-directed synthesis was performed in 50 µl solution containing 10 mM ImpA2′p5′A, 20 mM template, 30 mM $MgCl_2$, 0.2 M NaCl and 0.2 M *N*-ethylmorpholine buffer (pH 7.0) for 28 days at 0°C. (**B**) Reaction on [3′-5′]U_{10} template. The reaction was carried out under the same conditions as described above except for the template. (**C**) Control reaction without template. The reaction was performed under the same conditions as described above.

2′- or 3′-OH group of the ribosyl system takes part in efficient non-enzymatic template-directed condensation (23). Formation of compounds **I** and **II** indicates that ImpA2′p5′A reacts with the 2′- or 3′-terminal OH group of [2′-5′]U_{10} in the helix state. It is noteworthy that template-directed ligation takes place between the template and ligator strands in the 2′-5′ oligoribonucleotide system. No such ligation product was formed from ImpA2′p5′A and [3′-5′]U_{10}, although helix formation takes place in this system. The unique conformational geometry of the helix composed of [2′-5′]U_{10} and pA2′p5′A, as suggested by CD studies, may promote this unusual ligation reaction. The mechanism of the ligation reaction is not clear, however, one could propose a conceivable model of how the reaction might work. In the py-pu-py triplex model, the purine and the third strand are aligned in parallel and the two phosphodiester backbones are close together. This allows close proximity of the 2′,3′-OH of the template end with the activated 5′-end of a dimer unit bound to the last two bases of the template.

Previous studies demonstrate that 3′-5′-linked poly- or oligoribonucleotides serve as a template for oligomerization of complementary activated mononucleotides to form the corresponding oligoribonucleotides with 2′-5′ or 3′-5′ phosphodiester bonds (7,11–17). For example, polymerization of ImpA on 3′-5′-linked

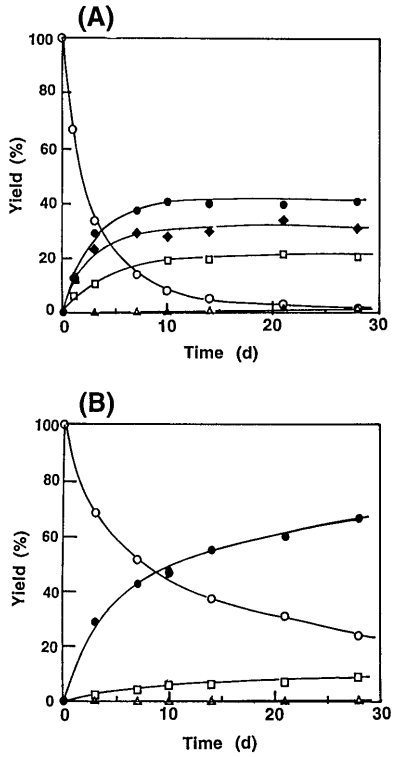


Figure 4. Time course of the template-directed synthesis. (**A**) Reaction on [2′-5′]U_{10} template. (**B**) Reaction on [3′-5′]U_{10} template. Yields were estimated from the peak area of the products on HPLC and are expressed based on the starting ImpA2′pA: ❍, ImpA2′p5′A; ●, pA2′p5′A; ❑, tetramer; △, hexamer; ◆, ligation products (**I** + **II**).

poly(U) or an oligo(U) template yields 2′-5′- and 3′-5′-linked oligo(A)s (14,24,25). The Pb^{2+}- or Zn^{2+}-catalyzed polymerization of ImpG on 3′-5′-linked poly(C) gives mainly 2′-5′- or 3′-5′-linked oligo(G)s respectively (15). 3′-5′-Linked poly- or oligo(C)s also promote template-directed polymerization of guanosine 5′-phosphor-2-methylimidazolide to yield oligo(G)s with mainly a 3′-5′ phosphodiester bond (11–14). The poly(U) template assists ligation of short-chain oligo(A)s to yield longer oligo(A)s with 2′-5′ and 3′-5′ phosphodiester bonds (26,27). Recently, Szostak *et al*. reported the non-enzymatic ligation of 3′-5′-linked heterooligoribonucleotides on the complementary 3′-5′-linked oligoribonucleotide template (28–30). Thus, non-enzymatic template-directed synthesis of oligonucleotides has been proved to proceed on 3′-5′-linked RNA templates. Very recently, Ertem and Ferris have reported that 2′-5′-linked oligo(C)s prepared from ImpC by montmorillonite catalysis serve as a template for formation of complementary oligo(G) from ImpG (18,19). The template ability of 2′-5′-linked oligo(C)s was also confirmed in the oligomerization of 2-MeImpG using hairpin templates (20). However, partially 2′-5′-linked oligo(U)s up to 9mer formed from ImpU by a montmorillonite catalyst failed in template-directed formation of oligo(A)s from ImpA, because hydrogen bonding in the A-U system is weaker than that in the G-C system and thus insufficient helix formation takes place between the oligo(U)s and ImpA (31). The current results establish that use of 2′-5′-linked diadenylate or longer oligoadenylates with a 2′-5′ or 3′-5′-linked oligo(U) template overcomes the above limitation of the A-U system in the template-directed chain elongation of oligoribonucleotides.

Table 1. Template-directed oligomerization of ImpA2′p5′A and ligation of ImpA2′p5′A to the template strand [2′-5′]U_{10}

Template	Product	Yield(%)[a]	Type of formed linkage (%)	
			2′-5′	3′-5′
[2′-5′]U_{10}	$(pA)_4$	21	81[b]	19[b]
	$(pA)_6$	1.5		
	I($U_{10}A_2$)	22	45[c]	55[c]
	II($U_{10}A_4$)	10	64[c]	36[c]
			35[d]	65[d]
[3′-5′]U_{10}	$(pA)_4$	8	94[b]	6[b]
	$(pA)_6$	0.5		

The reaction of ImpA2′p5′A (0.01 M) on the U_{10} template was run in the presence of 0.03 M $MgCl_2$ and 0.2 M NaCl in *N*-ethylmorpholine buffer (pH 7.0) at 0°C for 28 days.
[a]Yield was estimated from the peak area of products in the HPLC of the reaction mixture. The peak area of compounds **I** or **II** was allocated to the area due to [2′-5′]U_{10} and [2′-5′]A_2 or A_4 after correcting for their extinction coefficients. The yield data are expressed based on the starting ImpA2′p5′A.
[b]Ratio of type of linkage between two [2′-5′]$(pA)_2$ units, pA2′p**A**–**p**A2′pA.
[c]Ratio of type of linkage between [2′-5′]U_{10} and [2′-5′]$(pA)_2$ or $(pA)_4$.
[d]Ratio of type of linkage between [2′-5′]$U_{10}A_2$ and [2′-5′]$(pA)_2$.

In conclusion, the present finding demonstrates that long-chain 2′-5′-linked oligo(U)s formed by UO_2^{2+} ion-catalyzed polymerization of ImpU serves as a template for the template-directed synthesis of oligo(A)s from ImpA2′p5′A. [3′-5′]U_{10} also functions as a template for condensation of ImpA2′p5′A, but at lower efficiency compared with [2′-5′]U_{10}. The observation that 2′-5′-linked oligo(U) is more active than 3′-5′-linked oligo(U) as a template for oligomerization of ImpA2′pA is in contradiction of the finding by Prakash *et al.* that 3′-5′-linked oligo(C) is a more active template than the corresponding 2′-5′-linked oligo(C) for oligomerization of 2-MeImpG (20). The discrepancy is probably due to the fact the they used 2-MeImpG monomers rather than 2′-5′-linked dimers, ImpA2′pA, for template-directed oligomerization. Use of 2′-5′-linked RNAs as both the ligator strand and the template strand may promote template-directed oligomerization more efficiently than the chimeric system where 2′-5′-linked short oligoribonucleotides condense on 3′-5′-linked RNA templates. Joining of ImpA2′p5′A with the template [2′-5′]U_{10} also takes place to yield the longer oligonucleotides, which could also function as a template for other oligonucleotide syntheses. The 2′-5′-linked oligoribonucleotides are formed along with the 3′-5′-linked oligoribonucleotides by montmorillonite or metal ion catalysts or by any other model process of prebiotic synthesis of oligonucleotides. The 2′-5′- and 3′-5′-linked oligoribonucleotides could have formed a helix between complementary strands (22,32,33) and initiated template-directed ligation in the prebiotic era, prior to the advent of exclusively 3′-5′-linked RNA, which was selected due to its advantage of slow hydrolysis when the helix is formed (34) and/or of high helix forming ability (22,32,33) and conformational flexibility (21) compared with 2′-5′-linked RNA.

REFERENCES

1 Gilbert,W. (1986) *Nature*, **319**, 618.
2 Joyce,G.F. and Orgel,L.E. (1993) In Gesteland,R.F. and Atkins,J.F. (eds), *The RNA World*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 1–25.
3 Sawai,H. (1976) *J. Am. Chem. Soc*., **98**, 7037–7039.
4 Sawai,H. (1988) *J. Mol. Evol*., **27**, 181–186.
5 Sawai,H., Kuroda,K. and Hojo,T. (1989) *Bull. Chem. Soc. Jpn*., **62**, 2018–2023.
6 Sawai,H., Higa,K. and Kuroda,K. (1992) *J. Chem. Soc. Perkin Trans*., **I**, 505–508.
7 Orgel,L.E. and Lohrmann,R. (1973) *Acc. Chem. Res*., **7**, 368–377.
8 Ferris,J.P. and Ertem,G. (1992) *Science*, **257**,1387–1389.
9 Ferris,J.P. and Ertem,G. (1993) *J. Am. Chem. Soc*., **115**, 12270–12275.
10 Kawamura,K. and Ferris,J.P. (1994) *J. Am. Chem. Soc*., **116**, 7564–7572.
11 Inoue,T. and Orgel,L.E. (1981) *J. Am. Chem. Soc*., **103**, 7666–7667.
12 Inoue,T. and Orgel,L.E. (1981) *Science*, **219**, 859–862.
13 Joyce,G.F. (1987) *Cold Spring Harbor Symp. Quant. Biol*., **LII**, 41–51.
14 Orgel,L.E. (1995) *Acc. Chem. Res*., **28**, 109–118.
15 Lohrmann,R., Bridson,P.K. and Orgel,L.E. (1980) *Science*, **208**, 1464–1465.
16 Lohrmann,R. and Orgel,L.E. (1978) *Tetrahedron*, **34**, 853–855.
17 Orgel,L.E. (1992) *Nature*, **358**, 203–209.
18 Ertem,G. and Ferris,J.P. (1996) *Nature*, **379**, 238–240.
19 Ertem,G. and Ferris,J.P. (1997) *J. Am. Chem. Soc*., **119**, 7197–7201.
20 Prakash,T.P., Roberts,C. and Switzer,C. (1997) *Angew. Chem. Int. Edn English*, **36**, 1522–1523.
21 Sawai,H., Kuroda,K., Seki,J. and Ozaki,H. (1996) *Biopolymers*, **39**, 173–182.
22 Sawai,H., Seki,J. and Ozaki,H. (1996) *J. Biomol. Struct. Dynam*., **13**, 1043-1051.
23 Lohrmann,L.E. and Orgel,L.E. 1979) *J. Mol. Biol*., **113**, 193–198.
24 Sleeper,H.L., Lohrmann,R. and Orgel,L.E. (1979) *J. Mol. Evol*., **13**, 203–214.
25 Sawai,H. (1981) *J. Mol. Evol*., **17**, 48–51.
26 Lohrmann,L.E. and Orgel,L.E. (1979) *J. Mol. Evol*., **12**, 237–257.
27 Kanaya,E. and Yanagawa,H. (1986) *Biochemistry*, **25**, 7423–7430.
28 Bartel,D.P. and Szostak,J.W. (1993) *Science*, **261**, 1411–1418.
29 Rohatgi,R., Bartel,D.P. and Szostak,J.W. (1996) *J. Am. Chem. Soc*., **118**, 3332–3339.
30 Rohatgi,R., Bartel,D.P. and Szostak,J.W. (1996) *J. Am. Chem. Soc*., **118**, 3340–3344.
31 Ding,P.Z., Kawamura,K. and Ferris,J.P. (1996) *Origins Life Evol. Biosphere*, **26**, 151–171.
32 Giannaris,P G. and Damha,M.J. (1993) *Nucleic Acid Res*., **21**, 4742–4749.
33 Kierzek,R., He,L. and Turner,D.H. (1992) *Nucleic Acid Res*., **20**, 1685–1690.
34 Usher,D.A. and McHall,A.H. (1976) *Proc. Natl. Acad. Sci. USA*, **73**, 1149–1153.