Nonenzymatic recombination of RNA by means of transesterification

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Coupled nonenzymatic cleavage/ligation of oligoribonucleotides catalyzed by magnesium ions afforded longer RNA molecules with a new sequence. The efficiency of formation of ligation products reaches 6%. The possible role of this reaction in the evolution of the RNA world is discussed.

Key words: oligoribonucleotides, nonenzymatic ligation, catalysis.

It is assumed that in one of the steps of the prebiotic evolution, RNA molecules both transferred information encoded by the nucleotide sequence and functioned as the catalysts necessary for reproduction of information.[1,2] The RNA world encompasses steps of the chemical evolution from the simplest astrochemical reaction products to the formation of ensembles of long RNA molecules capable of exhibiting catalytic activity.[3—5]

The evolution of the RNA world proceeded *via* changes in the sequence and an increase in the molecular length, which should lead to the sophistication of the catalytic reactions and/or reactions where these RNAs play the key role. A possible mechanism enabling the formation of RNA molecules with a new sequence involves recombination processes based on the transphosphorylation.[6]

It is known[7,8] that 2´,3´-cyclophosphate produced upon the phosphodiester bond cleavage in RNA can be involved in the ligation with free OH groups of RNA. Earlier,[9,10] we have demonstrated that such metal ions as Mg^{2+}, Mn^{2+}, Co^{2+}, and Zn^{2+} substantially accelerate the reaction of 2´,3´-cyclophosphate with the 5´-OH group in oligonucleotides closely spaced due to hybridization on a complementary template. Presumably, analogous reactions proceed in complexes of partially complementary oligonucleotides. The probable mechanism involves 1) the formation of double helices with overhanging single-stranded fragments; 2) the phosphodiester bond cleavage in single-stranded regions and, as a consequence, the formation of a 2´,3´-cyclophosphate; 3) the formation of a new phosphodiester bond as a result of a reaction of the 2´,3´-cyclophosphate with the 5´-OH groups of the fragments closely spaced in the duplex. Both the cleavage and ligation are accelerated in the presence of divalent metal ions.[10,11] Thus, a new RNA molecule can be formed from several partially complementary RNA molecules by two successive nonenzymatic reactions.

From the standpoint of the energy consumption, the formation of an RNA molecule with a new sequence by recombination of the available molecules is more efficient because it does not require the involvement of additional reagents for the chemical activation of the 5´- and 3´-terminal groups, which is a prerequisite for all other nonenzymatic ligation and polymerization reactions (see the review[12]).

In the present study, we demonstrated the possibility of preparing RNA molecules with a new sequence by coupled cleavage and ligation reactions in a duplex of partially complementary oligonucleotides catalyzed by magnesium ions. These reactions are not only the simplest version of nonenzymatic recombination, but also serve as a model of the block replication, which could play the key role in the evolution of the RNA world.

Experimental

The oligoribonucleotides Lig (CUCUCUUCCUGAAAA) and Tem (GAGAGCAGGAA) used in the present study were synthesized according to the standard phosphoramidite method by the Research Group of Oligoribonucleotide Chemistry of the Laboratory of Nucleic Acid Chemistry of the Institute of Chemical Biology and Fundamental Medicine of the Siberian Branch of the Russian Academy of Sciences.

The [^{32}P] label was introduced at the 5´-end of the Lig oligonucleotide with the use of T4 polynucleotide kinase and [γ-^{32}P]-ATP according to a procedure described earlier.[13] The [^{32}P]-labeled Lig oligonucleotide (hereinafter, [^{32}P]-Lig) was isolated by denaturating gel electrophoresis in 20% polyacrylamide gel (PAG, acrylamide : bis-acrylamide = 19 : 1) in the presence of 8 *M* urea (hereinafter, dPAG). The oligonucleotide was dissolved in a 10 m*M* bis-Tris-propane—HCl (BTP) buffer, pH 7.0, containing 0.1 m*M* H_4edta and kept at –20 °C.

In all experiments, the reaction mixture (10 μL) containing the [^{32}P]-Lig, Lig, and Tem oligoribonucleotides (30 pmol each,

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3 μmol L^{-1}) in the BTP buffer (25 m*M*) at pH from 6.0 to 9.5 (hereinafter, the standard reaction mixture) was incubated under different conditions. The reaction was terminated by the addition of 3 *M* NaOAc (30 μL) and 96% EtOH (900 μL). The precipitates of the oligonucleotides were isolated by centrifugation, dissolved in a loading buffer (98% formamide, 10 m*M* Tris-HCl, pH 8.0, Bromophenol Blue (0.025%), and Xylene Cyanol (0.025%)), and analyzed in 20% dPAG. After electrophoresis, the gel was dried and the positions of radioactive products were determined on a Molecular Imager FX-PRO Plus instrument (Bio-Rad, USA). The quantitative results were obtained with the use of the Quantity One program. The yields of the reaction products were determined as the ratio of the intensity of the band corresponding to the product to the total intensity of the lane.

To study the pH dependence, 25 m*M* BTP buffers at pH 7.0, 7.5, 8.0, 8.5, 9.0, and 9.5 containing 5 m*M* $MgCl_2$ were used. In one series of experiments, the buffers contained also 50 m*M* NaCl. The samples were incubated at 37 °C for 3 days. Then the reactions were terminated, and the reaction products were analyzed as described above.

The influence of the temperature on the template-directed nonenzymatic cleavage/ligation of RNA was carried out in the standard reaction mixture containing 25 m*M* BTP, pH 9.5, and 5 m*M* $MgCl_2$. The samples were incubated at −20, 15, 20, 25, 30, 37, and 42 °C for 3 days.

To study the reaction kinetics, the standard reaction mixture containing 25 m*M* BTP, pH 9.5, and 5 m*M* $MgCl_2$ was incubated at 25 and 37 °C. The reactions were terminated at certain intervals of time, and the reaction products were analyzed in 20% dPAG as described above.

The influence of the concentration ratio between the Tem and Lig oligonucleotides on the yield of the cleavage/ligation products was determined in the reaction mixture (10 μL) containing the 25 m*M* BTP buffer, pH 9.0, 5 m*M* $MgCl_2$, 50 m*M* NaCl, and the Tem and Lig oligonucleotides. The concentration ratio between the latter ([Tem]/[Lig]) was varied from 0 to 25. The samples were incubated at 37 °C for 3 days. Then the reactions were terminated, and the reaction products were analyzed as described above.

To prepare a length marker (ladder), the 96-mer [^{32}P]-RNA HIV1* fragment was treated with 2 *M* imidazole at pH 7.0 in the presence of a carrier RNA as described in the study.[14] The resulting mixture of the fragments was used in the analysis of the cleavage/ligation products by gel electrophoresis in dPAG.

Results and Discussion

To study the coupled cleavage/ligation of RNA, we used a model system consisting of two partially complementary oligoribonucleotides Lig and Tem. The sequences of the oligonucleotides were chosen so that the 5′-region of the Tem oligonucleotide was complementary to the 5′-region of the Lig oligonucleotide, and the 3′-moiety of Lig was complementary to the 5′-region of Tem. The hybridization of these oligonucleotides will result in duplexes with different lengths. The tetraadenylate fragments of the Lig oligonucleotide are not involved in hybridization and form overhanging ends (Fig. 1, *a*). Data on the melting of the duplexes show that at pH 7.0 the melting temperature of the Lig/Tem complex is 48 °C and changes only slightly in the presence of NaCl. On the contrary, at pH 9.5 the melting temperature decreases to 28 °C in the presence of 50 m*M* NaCl and to 18 °C in the absence of NaCl. In spite of the theoretical possibility of the formation of long duplexes containing several molecules of the Lig and Tem oligonucleotides, such duplexes could not be detected by retention PAG under native conditions, which is apparently associated with their rather low stability (data not given).

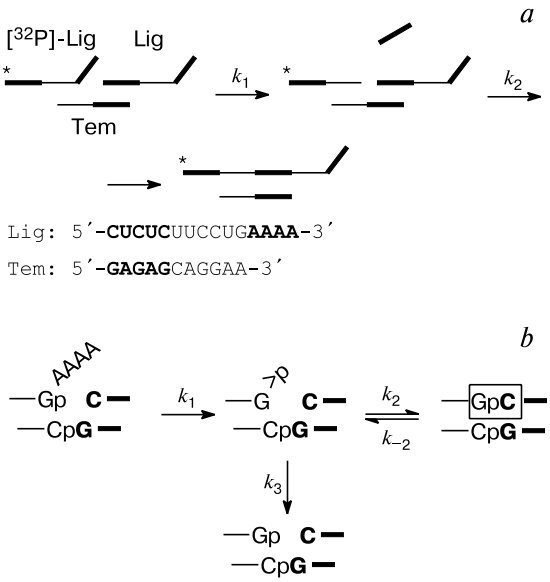

Fig. 1. (*a*) Scheme of the coupled cleavage/ligation reaction proceeding in the duplex of the Lig/Tem oligonucleotides. The ^{32}P radioactive label is marked with an asterisk. The complementary regions of the Lig and Tem oligonucleotides are graphically marked. (*b*) The scheme of the coupled cleavage/ligation of the Lig/Tem oligonucleotides. The reaction rate constants correspond to the following reactions: k_1, the phosphodiester bond cleavage in a single-stranded region, which is not involved in the duplex formation; k_2, the formation of a new phosphodiester bond (in a box) with the involvement of 2′,3′-cyclophosphate and the 5′-OH group of the second oligonucleotide molecule; k_{-2}, the cleavage of the newly formed phosphodiester bond; k_3, hydrolysis of 2′,3′-cyclophosphate giving rise to 2′- or 3′-phosphate.

To form the reaction complex, equal amounts of the unlabeled Lig oligonucleotide and the Tem oligonucleotide were added to the [5′-^{32}P]-Lig oligonucleotide. It should be noted that, due to the absence of the free 5′-hydroxy group, the [5′-^{32}P]-Lig oligonucleotide can be involved in the reaction only if it forms a duplex in a pair with unlabeled Lig and only if [^{32}P]-Lig is located in

* This fragment was kindly provided by A. V. Malyshev (Institute of Chemical Biology and Fundamental Medicine, Siberian Branch of the Russian Academy of Sciences).

the 5′-region of the complex (see Fig. 1). On the contrary, the unlabeled Lig oligonucleotide (5′-OH group) can occupy any position in the hybridization with the Tem oligonucleotide (see Fig. 1) and can be involved in the cleavage/ligation reaction regardless of the position. Thus, data on the yields of the cleavage/ligation products are underestimated because the [^{32}P]-Lig+[^{32}P]-Lig/Tem and Lig+[^{32}P]-Lig/Tem duplexes are nonproductive, and certain ligation products that are formed in the Lig+Lig/Tem duplex are not detected and are not taken into account because of the absence of the radioactive label.

The coupled cleavage/ligation reaction of the Lig oligonucleotide in the duplex with Tem was studied in the BTP buffer at different pH and temperatures in the presence or absence of NaCl with the use of Mg^{2+} ions as the catalyst. Unless otherwise stated, experiments were performed with the use of the standard reaction mixture containing equal concentrations of the oligonucleotides.

The pH dependence of the efficiency of the cleavage/ligation reaction of the Lig and Tem oligonucleotides in the presence of magnesium ions is presented in Fig. 2. It can be seen that the yield of the cleavage/ligation products increases with increasing pH. However, this is accompanied by an increase in the degree of RNA degradation. Since the monovalent metal ions influence the stability of duplexes due to a decrease in the energy of repulsion between the negatively charged phosphate groups, the pH dependence was studied both in the absence and presence of 50 m*M* NaCl. As can be seen from Fig. 2, the degree of oligonucleotide degradation decreases in

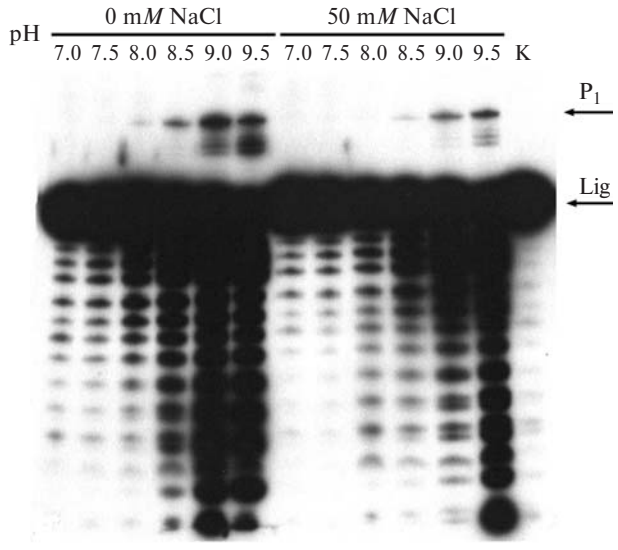

Fig. 2. Analysis of the products of the coupled cleavage/ligation reaction in 20% dPAG. The reaction was carried out in the presence of 5 m*M* $MgCl_2$ in a 25 m*M* BTP buffer at pH from 7.0 to 9.5 (pH are given in the upper line) at 37 °C for 3 days. The positions of the starting [^{32}P]-Lig oligonucleotide (lane K) and the ligation products, Lig and P_1, respectively are indicated by arrows.

the presence of NaCl; however, this is accompanied by a decrease in the yield of ligation products. The yields of ligation products in the absence of NaCl are 0.2, 1.1, and 2.5% at pH 8.5, 9.0, and 9.5, respectively. It should be emphasized that, although the free [^{32}P]-Lig oligonucleotide undergoes substantial degradation at pH >9 (higher than 85%), only the overhanging tetraadenylate sequence at the 3′-end of the ligation product undergoes degradation, whereas the phosphodiester bonds located inside the duplex remain intact (see Fig. 2, lanes 8.5, 9.0, and 9.5).

The incubation of the oligonucleotides at different pH and at different NaCl concentrations in the absence of magnesium ions does not result in detectable amounts of ligation products. Thus, the cleavage/ligation reaction proceeds only in the presence of the catalyst.

To investigate the influence of the temperature on the efficiency of formation of ligation products, we determined their yields in the temperature range from −20 to 42 °C (Fig. 3). These data show that an increase in the temperature leads to an increase in the degree of degradation of the [^{32}P]-Lig oligonucleotide. At temperatures below 30 °C, the cleavage occurs predominantly within the tetraadenylate residue; at higher temperatures, the phosphodiester bonds in the double-stranded region also undergo cleavage (see Fig. 3, *a*). The reaction product (P) appears even at 15 °C and is characterized by a single band, whose mobility in the gel corresponds to a 26-mer oligonucleotide. An increase in the temperature to 25 °C leads to an increase in the yield of ligation products, which give five bands in the gel. One of these bands corresponds to the full-size ligation product P; the other four bands, to products with the split terminal adenine residues (see Fig. 1, *a*). As the temperature is further increased, the yield of the ligation product decreased. In spite of the fact that all phosphodiester bonds in the [^{32}P]-Lig oligonucleotide undergo cleavage at 37 °C and pH 9.5, the total degree of degradation is ~88%, it is only the tetraadenylate region in the ligation product that is cleaved, whereas other bonds in the duplex remain intact. This is evidenced by the absence of bands in the gel between the ligation products in which all phosphodiester bonds in the overhanging oligoadenylate end are cleaved, and the starting [^{32}P]-Lig oligonucleotide. At 42 °C, the yield of ligation products is very low (≤0.1%), and the degree of degradation of the starting oligonucleotide is 93%.

An increase in the exposure time of the gel onto a Kodak FX screen of a Molecular Imager FX-PRO Plus instrument showed that longer RNA molecules (33—37-mer) are formed at temperatures from 20 to 37 °C, which corresponds to products of the cleavage/ligation reaction involving three molecules of the Lig oligonucleotide (see Fig. 3, *b*). The temperature dependence profile of the cleavage/ligation products with a max-

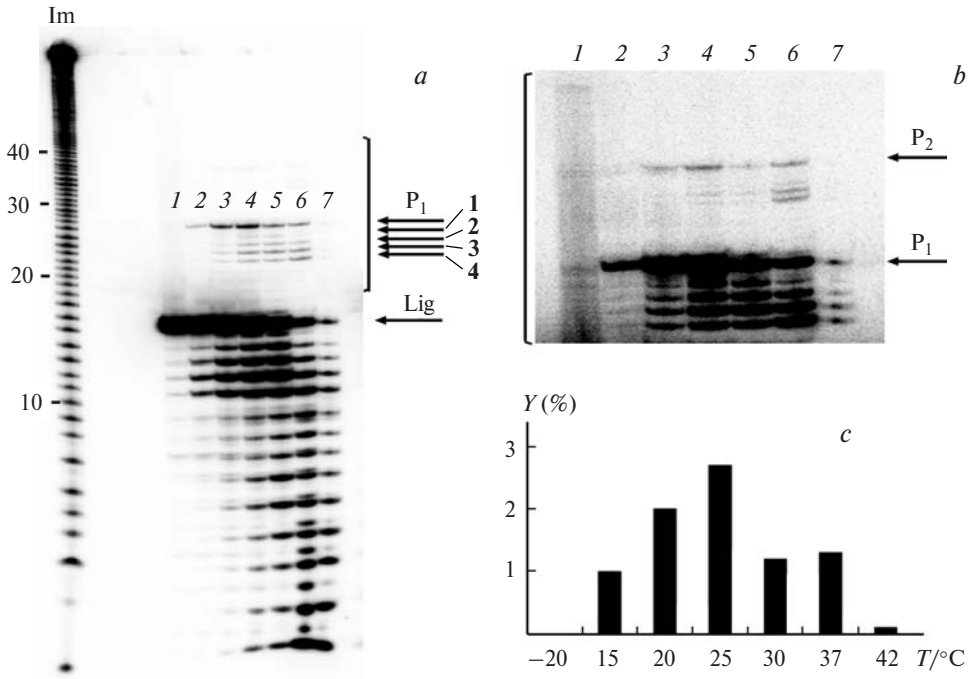

Fig. 3. Temperature dependence of the yield of the coupled cleavage/ligation products. (*a*) The separation of the cleavage/ligation products in 20% dPAG in the standard reaction mixture containing 25 m*M* BTP, pH 9.5, and 5 m*M* $MgCl_2$ at 20, 15, 20, 25, 30, 37, and 42 °C (lanes *1*—*7*, respectively) for 3 days. The position of the starting [^{32}P]-Lig oligonucleotide corresponds to Lig; the reaction products, to P_1, **1**, **2**, **3**, and **4**; lm is the ladder. (*b*) The longer-term exposure of the gel region presented in Fig. *a* (the region under investigation is indicated by a bracket in both parts of the figure). (*c*) The total yield (*Y*) of the coupled cleavage/ligation products (in % based on the starting amount of the oligonucleotide in the reaction mixture).

imum at 25 °C (see Fig. 3, *c*) correlates with the data on stability of the Lig/Tem duplex under the conditions used in the present study (m.p. 18 °C). According to these data, the percentage of the oligonucleotides involved in the duplex sharply decreases at temperatures above the melting temperature.

To elucidate the factors responsible for a decrease in the efficiency of formation of the ligation product as the temperature increases, the reaction kinetics was studied at 25 and 37 °C (Fig. 4). At 37 °C, the yield of the cleavage/ligation product increases during the first 30—45 h and then the yield decreases. As mentioned above, no products of the uniform cleavage of phosphodiester bonds in the reaction product P were observed. This is evidenced by the absence of bands in the gel between the starting [^{32}P]-Lig oligonucleotide and the ligation products (shown by a dashed line in Fig. 4, *a*), which is indicative of the selective cleavage of the newly formed phosphodiester

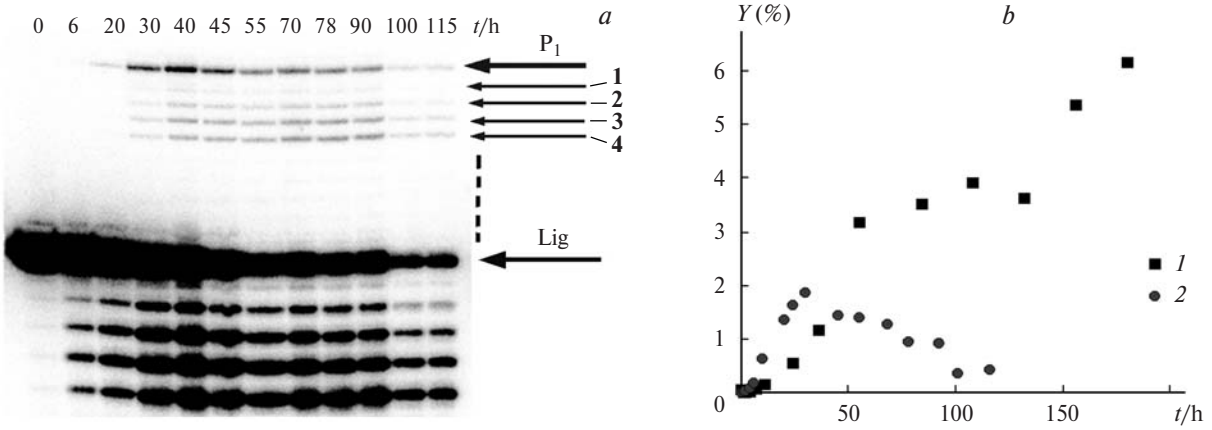

Fig. 4. Kinetics of accumulation of the coupled cleavage/ligation products. (*a*) The analysis of the ligation products at pH 9.5 and 37 °C in 20% dPAG. The position of the starting [^{32}P]-Lig oligonucleotide corresponds to Lig; the reaction products, to P_1, **1**, **2**, **3**, and **4**; (*b*) The accumulation of the ligation products at 25 (*1*) and 37 °C (*2*).

bond. At 25 °C, the yield of the reaction products linearly increases during 7.5 days and reaches 6% (see Fig. 4, *b*).

Since the stability of the ligation products depends on the time of existence in the duplex with the Tem oligonucleotide (RNA in a duplex is known[15] to be much more stable against cleavage), an increase in the concentration of the Tem oligonucleotide would be expected to lead to an increase in the yield of the reaction products. The dependence of the yield of the cleavage/ligation products on the concentration of the Tem oligonucleotide was studied for the Tem/[^{32}P]-Lig+Lig concentration ratios ranging from 0.04 to 25 in the reaction mixture containing 50 m*M* NaCl at pH 9.0 and 37 °C (Fig. 5). In the absence of the Tem oligonucleotide, as well as when it is deficient with respect to Lig, no ligation products were detected (see Fig. 5, lane 0). An increase in the concentration of the Tem oligonucleotide up to a 25-fold excess with respect to Lig (the concentration of Lig is always 3 μmol L^{-1}) does not lead to an increase in the yield of the reaction product. When the Tem/Lig concentration ratio becomes larger than 5, the degree of cleavage of Lig in the region complementary to Tem and involved in the duplex formation decreases, and the overhanging ends are predominantly cleaved (see Fig. 5, lanes with Tem/Lig = 5 and 25).

The secondary structure of the RNA molecules is formed through Watson-Crick hydrogen bonding between

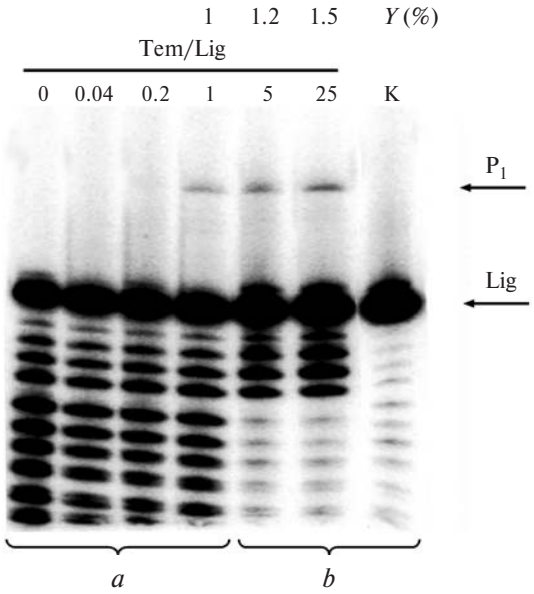

Fig. 5. Influence of the concentration of the Tem oligonucleotide on the yield of the coupled cleavage/ligation products. The autoradiograph of 20% dPAG after separation of the coupled cleavage/ligation products obtained at pH 9.0 and 37 °C. The Tem/Lig concentration ratios are given in the figure; the concentration of Lig is 3 μmol L^{-1}. The position of the starting [^{32}P]-Lig oligonucleotide (lane K) corresponds to Lig; the reaction product, to P_1; *a*, the cleavage of all bonds, the complex is not formed; *b*, the bonds in the complex are intact.

the completely or partially complementary regions. Interactions between partially complementary sequences of RNA produce loops of different sizes, in which, like in single-stranded RNAs, the phosphodiester bonds undergo transesterification much more easily than in double-stranded regions.[11] The cleavage of the phosphodiester bonds between nucleotides in loops or in a single-stranded region gives rise to duplexes containing single-stranded gaps. Stacking interactions between bases adjacent to the gap site cause a decrease in the free energy of the duplex by 1.5—2.5 kcal mol^{-1}.[16] Thus, the cleavage of noncomplementary nucleotides in an imperfect double helix can be considered as a transition of the system to a more stable state in which all or almost all phosphodiester bonds are involved in the duplex and are cleaved at a low rate.

In our experimental system consisting of the Lig and Tem oligonucleotides, the above-described duplex is formed upon cleavage of the tetraadenylate fragment, which is not involved in the duplex formation. The resulting 2′,3′-cyclophosphate can be either involved in the nonenzymatic ligation reaction with the 5′-OH group adjacent to the Lig oligonucleotide to form a new RNA molecule or undergo hydrolysis to give the terminal phosphate group, which is not involved in the ligation (see Fig. 1, *b*). The coupled cleavage/ligation products consist of two (or more) fragments of the Lig oligonucleotide complementary to the Tem oligonucleotide and contain from one to four adenine residues at the 3′-end. An increase in the molecular length leads to an increase in stability of the duplex with the Tem oligonucleotide and, consequently, to an increase in stability of the phosphodiester bonds in the duplex.

In our experiments, the yield of the ligation product was determined after short-term incubation, when only a small amount of the starting oligonucleotide was consumed, *i.e.*, we analyzed the initial step of the reaction. In this case, the efficiency of the reaction under the experimental conditions can be estimated by comparing the yields of the reaction product. In the cases where a large amount of the [^{32}P]-Lig oligonucleotide was consumed (for example, at temperatures higher than 30 °C, see Fig. 3, *a*), we studied the reaction kinetics as well.

A decrease in stability of the Lig/Tem duplex as pH increases from 7.0 to 9.5 is attributed to a decrease in the number of hydrogen bonds due to deprotonation of the heterocyclic bases. The stabilizing effect of the sodium ions shielding the negative charges of the phosphate groups is more pronounced at pH 9.5. The observed increase in the yield of the cleavage/ligation product with increasing pH is accounted for by an increase in the concentration of the aqua hydroxo complex with the magnesium ion, $Mg[(H_2O)_5(OH)]^+$. In the presence of NaCl, an increase in stability of the Lig/Tem duplex leads to a decrease in the degree of cleavage of the starting [^{32}P]-Lig oligoribo-

nucleotide (see Fig. 2). The observed decrease in the yield of the cleavage/ligation product can be associated with a decrease in the accessibility of the corresponding phosphodiester bond of GpA to the attack by the aqua hydroxo complex of the metal ion. Earlier, we have studied the dependence of the yield of the reaction product on the NaCl concentration in the range from 5 mmol L^{-1} to 5 mol L^{-1} and found that an increase in the NaCl concentration causes a substantial decrease in both the degradation of the starting oligonucleotide and the yield of the reaction product (unpublished data). Thus, the competition between the Na^+ and $[Mg(H_2O)_5(OH)]^+$ ions for the binding sites with RNA can serve as an alternative explanation of the decrease in the efficiency of transesterification in the presence of NaCl.

An increase in the concentration of the Tem oligonucleotide should lead to a decrease in the concentration of the free Lig oligonucleotide due to the formation of the duplex between these oligonucleotides. The reaction does not proceed in the absence of the Tem oligonucleotide, and the formation of the product is observed only in reactions with an equimolar ratio of the oligonucleotides, the yield of the product changes only slightly as the concentration of Tem is increased. In the resulting duplex, the phosphodiester bonds are stabilized. The reason for the observed stabilization of the bonds and the absence of an increase in the yield of the product is attributed to the fact that for the cleavage/ligation reaction to occur, the [Lig+Lig/Tem] duplex is required, whereas the formation of the [Lig/Tem] duplex is sufficient for stabilization.

An increase in the temperature in the experimental system under consideration can influence the efficiency of the reaction in the following two ways: by a decrease in stability of the duplex and an increase in the rate of both cleavage and ligation of phosphodiester bonds. The opposite influence of the above-mentioned factors provide evidence that there are conditions providing the highest efficiency of formation of the coupled cleavage/ligation product. This assumption is confirmed by the temperature dependence of the yield of the reaction product (see Fig. 3, *c*). Indeed, the highest yield of the product is observed at 25 °C in spite of the fact that the oligonucleotides exist predominantly in the free state (T_{melt} 18 °C, pH 9.5), the reaction product being gradually accumulated over 7 days. At 37 °C, the amount of the reaction products increased more rapidly than at 25 °C (see Fig. 4, *a*). However, the amount of the reaction product decreased after 40—45 h. The degradation of the Lig oligonucleotide at this instant is at least 50%, all phosphodiester bonds in the oligonucleotide that exists in solution in the free state being involved. The coupled cleavage/ligation product forms a more stable duplex with Tem and, as a consequence, it does not undergo cleavage (see Fig. 4, *b*).

The absence of a uniform cleavage profile for the cleavage/ligation product at 37 °C is indicative of the selectivity of phosphodiester bond cleavage. If the cleavage/ligation reaction affords the 3′,5′-phosphodiester linkage, all phosphodiester bonds within the double helix in the duplex formed by the reaction product and the Tem oligonucleotide should be equally stable against cleavage. The 3′,5′-phosphodiester linkages in the RNA—RNA duplexes are known to exist in the conformation that prevents transesterification, whereas the 2′,5′-phosphodiester linkages readily adopt the in-line conformation necessary for the nucleophilic attack.[7,17] Therefore, in the case of formation of the 3′,5′-phosphodiester linkage, the amount of the reaction product can decrease exclusively as a result of phosphodiester bond cleavage in the ligation product upon its elimination from the duplex with the oligonucleotide template. In this case, a uniform profile of phosphodiester bond cleavage would be expected. However, this was not experimentally observed.

It can be concluded that the observed nonuniform profile of phosphodiester bond cleavage in the ligation product corresponds to the cleavage of the 2′,5′-phosphodiester linkage, which is formed in the cleavage/ligation reaction and is substantially less stable against cleavage than the 3′,5′-phosphodiester linkages in the duplex. This conclusion is consistent with the published data[7,10,18—20] on the predominant formation of 2′,5′-phosphodiester linkages in the template-directed ligation of 2′,3′-cyclophosphate.

To summarize, we demonstrated that the coupled cleavage/ligation reaction catalyzed by magnesium ions affords longer RNA molecules with a new sequence from unstable duplexes of partially complementary RNAs. The reaction gives the product in a yield of up to 6% at micromolar concentrations of oligonucleotides and even under conditions where the degree of hybridization of oligonucleotides is at most 50%. The results of the present study confirmed the possibility of the nonenzymatic recombinant processes between RNA molecules as the coupled cleavage/ligation reactions. These processes could play an important role in the prebiotic evolution of RNA.

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References

1. W. Gilbert, *Nature,* 1986, **319**, 618.
2. G. F. Joyce and L. E. Orgel, *The RNA World*, Eds R. F. Gesteland and J. F. Atkins, Cold Spring Harbor Press, New York, 1999, 48.

3. J. P. Dworkin, A. Lazcano, and S. L. Miller, *J. Theor. Biol.*, 2003, **222**, 127.
4. L. E. Orgel, *Orig. Life. Evol. Biosph.*, 2003, **33**, 211.
5. A. S. Spirin, *Mol. Biol.*, 2005, **39**, 466 [*Mol. Biol.*, 2005, **39**, 550 (Engl. Transl.)].
6. A. B. Chetverin, *FEBS Lett.*, 1999, **460**, 1.
7. D. A. Usher and A. H. McHale, *Science*, 1976, **192**, 53.
8. D. Lafontaine, D. Beaudry, P. Marquis, and J. P. Perreault, *Virology*, 1995, **212**, 705.
9. A. V. Lutay, E. L. Chernolovskaya, M. A. Zenkova, and V. V. Vlassov, *Dokl. Akad. Nauk*, **401**, 163 [*Dokl. Biochem. Biophys.,* 2005, **401**, 705 (Engl. Transl.)].
10. A. V. Lutay, E. L. Chernolovskaya, M. A. Zenkova, and V. V. Vlassov, *Biogeosciences*, 2006, **3**, 243.
11. D. Husken, G. Goodall, M. J. Blommers, W. Jahnke, J. Hall, R. Haner, and H. E. Moser, *Biochemistry*, 1996, **35**, 16591.
12. L. E. Orgel, *Crit. Rev. Biochem. Mol. Biol.*, 2004, **39**, 99.
13. M. Silberklang, A. Prochiantz, A. L. Haenni, and U. L. Rajbhandary, *Eur. J. Biochem.*, 1977, **72**, 465.
14. A. V. Vlassov, V. V. Vlassov, and R. Giege, *Dokl. Akad. Nauk*, 1996, **349**, 411 [*Dokl. Biochem. Biophys.*, 1996 **349** (Engl. Transl.)].
15. G. A. Soukup and R. R. Breaker, *RNA*, 1999, **5**, 1308.
16. M. J. Lane, T. Paner, I. Kashin, B. D. Faldasz, B. Li, F. J. Gallo, and A. S. Benight, *Nucleic Acids Res.*, 1997, **25**, 611.
17. R. Rohatgi, D. P. Bartel, and J. W. Szostak, *J. Am. Chem. Soc.*, 1996, **118**, 3340.
18. S. Uesugi and M. Ikehara, *Biochemistry*, 1977, **16**, 493.
19. F. Cote and J. P. Perreault, *J. Mol. Biol.*, 1997, **273**, 533.
20. A. Flynn-Charlebois, T. K. Prior, K. A. Hoadley, and S. K. Silverman, *J. Am. Chem. Soc.,* 2003, **125**, 5346.

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