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Ribonucleic Acid Ligase Activity of Deoxyribonucleic Acid Ligase from Phage T4 Infected *Escherichia coli*†

Hiroshi Sano and Günter Feix*

ABSTRACT: This study presents evidence that the DNA ligase of phage T4 purified according to the procedure of Weiss *et al.* (Weiss, B., Jacquemin-Sablon, A., Live, T. R., Fareed, G. C., and Richardson, C. C. (1968), *J. Biol. Chem. 243*, 4543) can be used as an RNA ligase. Working with high enzyme concentrations, with short oligoribonucleotides, and at 0°C, oligo(rU), oligo(rI), and oligo(rC) could be joined head-to-tail, respectively, in the presence of the corresponding complementary polyribonucleotides. Oligo(rA), however, in combination with poly(rU) or oligo(rU) could not be covalently linked under the conditions used.

Deoxyribonucleic acid ligase activities are implicated in replication, repair, and most probably also in recombination of double-stranded DNA in procaryotic and eukaryotic cells (Klein and Bonhoeffer, 1972; Radding, 1973). They catalyze the formation of phosphodiester bonds between the 5′-phosphoryl group and the 3′-hydroxyl group of nicks (interruptions of one strand) in double-stranded DNA structures (Richardson, 1969). Because of this activity, DNA ligases have become helpful tools in several synthetic reactions *in vitro* (Goulian *et al.*, 1967; Agarwal *et al.*, 1970; Lobban and Kaiser, 1973). Recently, it was found that the DNA ligase of phage T4, a polynucleotide ligase studied rather extensively, will also catalyze the end-to-end joining of bihelical DNA with fully base paired termini (Sagaramella and Khorana, 1972). In addition to this reaction, DNA ligase of phage T4 will also accept DNA-RNA hybrid structures as substrates, which has been demonstrated by the head-to-tail joining of various oligodeoxyribonucleotides on a polyribonucleotide template and *vice versa* (Kleppe *et al.*, 1970; Fareed *et al.*, 1971). Similar experiments, however, using oligo(rA)-poly(rU) or oligo(rU)-poly(rA)[1] as substrate combinations were unsuccessful (Kleppe *et al.*, 1970; Fareed *et al.*, 1971), and led to the conclusion that DNA ligase of phage T4 might not work with double-stranded RNA structures as substrate. During our attempts to connect RNA molecules covalently with each other, we restudied the DNA ligase of phage T4 and found that this enzyme can indeed be used as an RNA ligase if RNA-RNA structures other than oligo(rA)-poly(rU) are offered as substrates. It will be shown that under the conditions used (high enzyme concentration, rather short oligonucleotides, low temperature) DNA ligase of T4 does behave as an RNA ligase connecting oligoribonucleotides in various RNA-RNA combinations in a head-to-tail manner with the only exception that of oligo(rA)-poly(rU).

Experimental Section

Materials

DNA ligase was prepared principally according to the method described by Weiss *et al.* (1968) with a modification introduced by Knopf (1974). T4 am 4647 infected *Escherichia coli* strain B41 cells (50 g) (detailed procedure will be given elsewhere) were homogenized, treated by streptomycin, fractionated with ammonium sulfate, dialyzed, and loaded on a DEAE-cellulose column (4.5 × 11 cm) equilibrated with 10 mM Tris-HCl (pH 7.4)-1 mM β-mercaptoethanol (TM buffer), and eluted with 1000 ml of a linear gradient from 0 to 0.3 M sodium chloride in TM

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[1] The abbreviations used are specified in *Biochemistry 9*, 4022 (1970).

buffer (Figure 1A). The indicated fractions of peak I of the DEAE-cellulose columns from several batches were pooled, dialyzed, applied to a phosphocellulose column (3 × 10 cm), and eluted with 500 ml of a linear gradient from 0 to 0.6 M sodium chloride in TM buffer (Figure 1B). Active fractions detected by the ATP-PP_i exchange assay (see below) were combined, dialyzed first against TM buffer, and then against a saturated ammonium sulfate solution in TM buffer. The precipitate was collected by centrifugation and dissolved in 60% glycerol-TM buffer. The specific activity of this solution was 700 units/ml (1 unit = 10^{-9} mol of $^{32}PP_i$ incorporated/20 min at 37°). The apparent Michaelis constant for ATP as estimated by ATP-PP_i exchange reaction was found to be 1.0×10^{-5} M.

Polynucleotide kinase was prepared by the method described by Richardson (1965) with a modification. Details of this procedure and properties of the enzyme isolated will be reported elsewhere. The specific activity was 200 units/ml.

Alkaline phosphatase from *E. coli* was purchased from Boehringer Co. Ltd. (15429 EPBG, 1 mg/ml). This sample was free of RNase as found by sedimentation analysis of phosphatase treated Qβ-RNA.

Polynucleotides. Poly(rA), poly(rI), poly(rC), and poly(rU) were purchased from Boehringer Co. Ltd. Poly(dT) was prepared according to the method described by Kato *et al.* (1967).

Oligonucleotides. Oligo(rI)$_5$ (from Miles) and oligo(rA)$_{10}$ (from Boehringer) were purchased. Oligo(rU)$_{12-18}$ was prepared by partial alkaline hydrolysis of poly(rU) (Simpkins and Richards, 1967). Poly(rU) (38 mg) in 10 ml of 0.1 M potassium hydroxide was incubated for 15 min at 37°, treated with 0.1 N HCl to open 3′-2′ cyclic phosphates, neutralized, and fractionated by DEAE-Sephadex-A-25 chromatography in the presence of 7 M urea. The fractions containing oligonucleotides of chain lengths from 12 to 18 nucleotides as estimated by the elution profile were pooled, dialyzed, and incubated with alkaline phosphatase (5 μg/25 OD units) for 3 hr at 37°.

The reaction mixtures were fractionated on a Sephadex G-75 column equilibrated with 20 mM Tris-HCl buffer (pH 7.4). The oligomer-containing fractions were pooled, dried by Evapomix evaporation, taken up in 0.1 ml of H_2O, and incubated with 0.2% diethyl pyrocarbonate for 30 min at 30°. After destroying the remaining diethyl pyrocarbonate by heating, the sample was ready for phosphorylation by polynucleotide kinase. Oligo(rC)$_{15-20}$ was prepared from poly(rC) in a similar manner; details of the procedure will be reported elsewhere. Chain lengths were determined by the ratio of A_{260} units to 5′-terminal ^{32}P after phosphorylation by polynucleotide kinase.

Other materials. [γ-^{32}P]ATP was prepared by the method described by Glynn and Chappell (1964) with a modification by Kühn of this department. The specific activity was 1.04×10^{10} cpm/μmol. Whatman DE 52 (DEAE-cellulose) was used without precycling and Whatman P 11 (phosphocellulose) was washed extensively with alkali and acid before use. Salts were reagent grade from Merck (Germany).

Methods

Phosphorylation of Oligomers with [γ-^{32}P]ATP. A 200-μl reaction mixture contained 0.1 M Tris-HCl buffer (pH 8.0), 10 mM magnesium chloride, 6 mM dithiothreitol, 0.5 mM [γ-^{32}P]ATP, 15 μl of polynucleotide kinase (3.5 units), and various concentrations of oligonucleotides (3–10 optical density (OD) units). After incubation for 30 min at 30°, the reaction mixture was applied to a Sephadex G-25 column (0.6 × 100 cm) and eluted with 20 mM triethylammonium bicarbonate (pH 7.0). The first radioactive fractions as detected by Cerenkov radiation were pooled, dried by Evapomix evaporation, and dissolved in 100 μl of water. The specific activity of each oligomer is given in the figure legends.

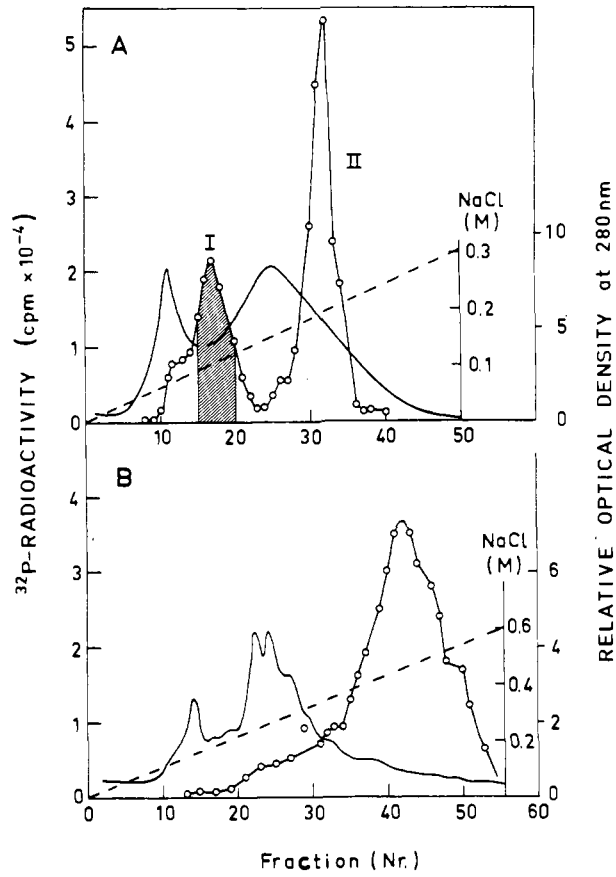

FIGURE 1: Chromatographies of DNA ligase on DEAE-cellulose (A) and phosphocellulose (B). Fractions of the shaded area of peak I from several parallel DEAE-cellulose columns were pooled and applied to the phosphocellulose column. Optical density was checked at 280 nm by a spectrophotometer Uvicord II (—). Enzymatic activity was measured with an aliquot of 50 μl by the ATP-PP_i exchange reaction (○).

Assay for ATP-PP_i Exchange Activity of DNA Ligase. A 150-μl reaction mixture contained 0.1 M Tris-HCl (pH 7.6), 10 mM $MgCl_2$, 5 μg of bovine serum albumin, 6 mM dithiothreitol, 10 mM ATP, 40 mM $^{32}PP_i$ (sp act. 46.3 mCi/mmol), and 50-μl aliquots from each fraction eluted from DEAE-cellulose and PC-cellulose chromatography. After 20 min of incubation at 37°, 2 ml of Norit solution was added to the sample. The suspension was put on a glass fiber filter, washed with cold 0.01 N HCl, dried, and counted for radioactivity.

Assay of Joining by DNA Ligase. A 100-μl reaction mixture contained 0.1 M Tris-HCl buffer (pH 7.6), 10 mM magnesium chloride, 6 mM dithiothreitol, 50 μg of bovine serum albumin (RNase free, treated with iodoacetate (Yarus and Rashbaum, 1972)), 1 mM ATP, 35 units of DNA ligase, and poly- and oligonucleotides in concentrations as listed in the figure legends. Before mixing with the other components, the poly- and oligonucleotides were preincubated in 0.1 M Tris-HCl buffer first for 5 min at 45°, then for 10 min at 30°, and finally for 60 min at 10°.

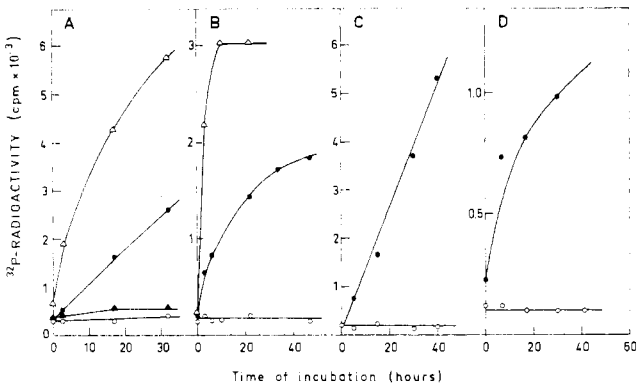

FIGURE 2: Kinetics of T4 ligase catalyzed joining of various oligoribonucleotides in the presence of corresponding polyribonucleotides. (A) Joining of oligo(rU) on poly(rA). The reaction mixture (see Experimental Section) contained 1.2 mM poly(rA) and 1.2 mM [5′-^{32}P]oligo(rU) (sp act. 5.5 × 10^6 cpm/μmol). (△) Reactions with additional 0.2 M NaCl (●), with additional 1.0 M NaCl (▲), without ligase (○), and without poly(rA) (○) are also shown. (B) Joining of oligo(rA) on poly(rU). The reaction mixture contained 0.75 mM poly(rU) and 0.74 mM [5′-^{32}P]oligo(rA) (sp act., 1.3 × 10^7 cpm/μmol) with ligase (●), without ligase (○), and without poly(rU) (○). As a control, joining of oligo(rA) on poly(dT) is also shown (△). The reaction mixture contained 20 μM poly(dT) and 37 μM oligo(rA). (C) Joining of oligo(rI) on poly(rC). The reaction mixture contained 1.4 mM poly(rC) and 1.5 mM [5′-^{32}P]oligo(rI) (sp act., 1.6 × 10^6 cpm/μmol) with ligase (●), without ligase (○), and without poly(rC) (○). (D) Joining of oligo(rC) on poly(rI). The reaction mixture contained 0.16 mM poly(rI) and 0.14 mM [5′-^{32}P]oligo(rC) (sp act., 4.3 × 10^6 cpm/μmol) with ligase (●), without ligase (○), and without poly(rI) (○).

The reaction mixture was incubated at 0° for various periods of time. A 10-μl aliquot was mixed with 0.1 ml of cold 0.1 M Tris-HCl buffer (pH 8.0), containing 5 μg of alkaline phosphatase, incubated for 30 min at 62–65°, mixed with 2 ml of Norit solution (20 g of Norit, 250 ml of 1 N HCl, 112 mg of sodium pyrophosphate, and 35 mg of sodium dihydrogen phosphate per l.), and applied on a Whatman glass fiber filter paper GF/C, washed several times with cold 0.01 N HCl, dried, and counted for radioactivity.

Column Chromatography. A 50-μl sample of the ligase reaction mixture was mixed with 0.2 ml of 0.1 M Tris-HCl buffer (pH 8.0), containing 10 μg of alkaline phosphatase, incubated for 30 min at 65°, and then fractionated using a combination column (0.6 × 140 cm) of Sephadex G-75 (upper phase 55 cm) and Bio-Gel A 1.5 m (lower phase 85 cm) (Stavrianopoulos *et al.*, 1972), which was equilibrated with 0.1 M triethylammonium bicarbonate (pH 7.0). The column was operated at 20° and 0.65-ml fractions were collected. Appropriate fractions as analyzed by Cerenkov radiation measurement were pooled, dried by Evapomix evaporation, and dissolved in 50 μl of water for final use.

Paper Chromatography and Paper Electrophoresis. For these analyses, Schleicher and Schüll paper, No. 2040b, was used. Before spotting the material, the starting area was treated with 0.4 M EDTA and dried. High voltage electrophoresis was carried out in 0.15 N ammonium acetate (pH 3.5) at 2000 V in the Savant apparatus (Model No. LT 20 A). Paper chromatography was carried out in descending fashion in the system 1-propanol–concentrated ammonia–distilled water (55:10:35, v/v) for 10–15 hr. The paper was dried, cut into 1-cm strips, and counted for radioactivity.

Optical Density Measurements. Concentrations of materials were calculated based on the molar extinction coefficient of corresponding mononucleotides. Hypochromicity effects of the polymers were not included in the calculations.

Measurement of Radioactivity. All radioactive determinations were made in a Beckman liquid scintillation spectrophotometer using a toluene based scintillator.

Results

This study originated from our plan to connect rather short oligoribonucleotides with each other for synthetic purposes. Therefore, all ligase incubations throughout this work were performed at 0°.

Oligoribonucleotides having free 5′- and 3′-hydroxy groups were phosphorylated at the 5′ end with [^{32}P]phosphate by catalysis with polynucleotide kinase. These ^{32}P-labeled oligoribonucleotides were mixed with the corresponding complementary polyribonucleotides in equal amounts in terms of mononucleotides and incubated with DNA ligase at 0° under conditions described in the legend of Figure 2. At the times indicated in the figure, 10-μl samples were taken and analyzed for alkaline phosphatase resistant ^{32}P radioactivity. It can be seen that all oligoribonucleotides tested (oligo(rA), oligo(rU), oligo(rI), and oligo(rC)) became increasingly alkaline phosphatase resistant after prolonged times of incubation suggesting a sealing effect of DNA ligase. Oligonucleotides incubated without ligase remained completely alkaline phosphatase sensitive. The experiment with oligo(rA) and poly(dT) as substrates, incubated under the same conditions, is included in the figure for comparison as a very efficient DNA ligase substrate (Figure 2B). In this particular series of experiments 9% of input oligo(rU), 38% of input oligo(rI), 13% of input oligo(rC), and 1.8% of input oligo(rA) became alkaline phosphatase resistant after 20 hr of incubation at 0° (see Table I).

The low incubation temperature used is favorable for the formation of double-stranded RNA and also helps to protect against contaminating traces of RNAse activity which cannot be detected at this low incubation temperature as tested by sedimentation analysis of incubated ^{14}C-labeled phage RNA. Increase of the salt concentration in the incubation mix in order to facilitate the formation of double-stranded RNA lowered the rate of reaction (Figure 2A) in agreement with the results of Harvey and Wright (1972), who studied the oligo(dT)–poly(dA) system in DNA ligase reactions.

It should be mentioned that similar experiments with the substrate combinations oligo(rG)–poly(rC) or oligo(rC)–poly(rG) turned out to be rather difficult since such oligomer–polymer combinations formed alkaline phosphatase resistant complexes during the prolonged times of incubation at low temperature even in the absence of DNA ligase. This may reflect the special behavior of guanylic acid containing polymers.

In order to separate the alkaline phosphatase resistant product from the starting material for further analysis and to get an estimate of the product size, alkaline phosphatase treated incubation mixtures were passed through a combination column of Sephadex G-75–Bio-Gel A 1.5 m. This type of column was devised to concomitantly separate a wide range of different sized oligo- and polynucleotides (Stavrianopoulos *et al.*, 1972). The elution profiles of Figure 3 show that only in the case of the substrate combination oligo(rA)–poly(dT) was a long product formed (Figure 3B). All the other reactions yield heterogeneous, rather short products which are only slightly larger than the un-

TABLE I: Polynucleotide Ligase Activity.[a]

Poly-	[5′-^{32}P]Oligo-	^{32}P cpm of 10 μl of React. Mixture		^{32}P cpm of 3′(2′)-Mononucleotides Recovered from 10 μl of React. Mixture[b]	Ratio of APase Resistant ^{32}P cpm to Input ^{32}P cpm (%)
		Input	APase Resistant		
(dT)	$(rA)_{10}$	2.6×10^3	2.8×10^3	7.1×10^2	100
(rA)	$(dT)_{12-18}$	2.5×10^3	1.4×10^3		60[c]
(rU)	$(rA)_{10}$	1.0×10^5	1.8×10^3	0	1.8
(rA)	$(rU)_{12-18}$	6.6×10^4	5.8×10^3	5.3×10^3	9
(rC)	$(rI)_5$	7.2×10^4	2.7×10^4	2.5×10^4	38[d]
(rI)	$(rC)_{15-20}$	6.0×10^2	5.4×10^2	5.4×10^2	13

[a] Reaction conditions: 20 hr at 0°; 3.5 units of ligase/10 μl of reaction mixture; oligonucleotide and polynucleotide concentrations as in Figure 2. [b] Hydrolysis to [3′(2′)-^{32}P]mononucleotide: the remaining 50 μl of the reaction mixture, after the kinetic analysis, was digested with alkaline phosphatase at 65°, fractionated by the combination column of Sephadex G75 and Bio-Gel A 1.5 m, hydrolyzed completely with 0.2 N KOH for 18 hr at 37°, neutralized with a Dowex column (pyridine form), dried by Evapomix evaporation, and used for high voltage electrophoresis. [c] 6 hr at 4°; 2.1 units of ligase/10 μl of reacting mixture. [d] Different experiment from that shown in the figures.

reacted oligonucleotide substrates tested in separate runnings of the same column (indicated in the figure by closed circles). The slower moving peak represents inorganic [^{32}P]phosphate released from unreacted oligonucleotide. Since the combination column will not differentiate effectively between short oligonucleotides of different sizes, the product size of the oligo(rI)-poly(rC) reaction was further characterized as an example. The analysis on a DEAE-cellulose column under the conditions of Tener (1967) and on a calibrated G-25 Sephadex column revealed that the product consists mostly of dimers and a few higher oligomers of the starting oligo(rI) (data not shown). It is not surprising that the product size did not increase more, because a successful ligase reaction will occur only if the oligonucleotides are direct neighbors on the template polynucleotide. This apparently did not happen very often under the conditions used since the incubation mixtures did not contain an excess of oligonucleotides. The rather long product of the oligo(rA)-(dT) reaction (Figure 3B), however, was possibly formed for the following reasons. The ratio of oligonucleotide to polynucleotide used may be more favorable for the hybrid formation, the enzyme may prefer the DNA-RNA hybrid as substrate, the oligo(rA) may creep rather good along the poly(dT) chain.

In order to verify that the analysis of alkaline phosphatase resistant ^{32}P radioactivity reflected the formation of phosphodiester bonds we looked for the transfer of ^{32}P radioactivity from the 5′ end of the starting oligonucleotide to the 3′ end of monophosphate nucleotides after a complete alkaline hydrolysis of the reaction product. The ^{32}P radioactivity of the 5′-terminal nucleotides of unreacted oligonucleotides could be found in nucleotide diphosphates after the alkaline hydrolysis. Fractions of the column chromatography (Figure 3) belonging to the peak of alkaline phosphatase resistant ^{32}P radioactivity were pooled, incubated for 18 hr in 0.2 M KOH, and, after neutralizing and concentrating, subjected to high voltage paper electrophoresis at pH 3.5. The radioactivity profiles of these electropherograms, depicted in Figure 4, show that in the case of oligo(rU), oligo(rC), and oligo(rI) as starting oligonucleotides, the main peak of radioactivity corresponds to the position of the mononucleotides run the same paper as optical density

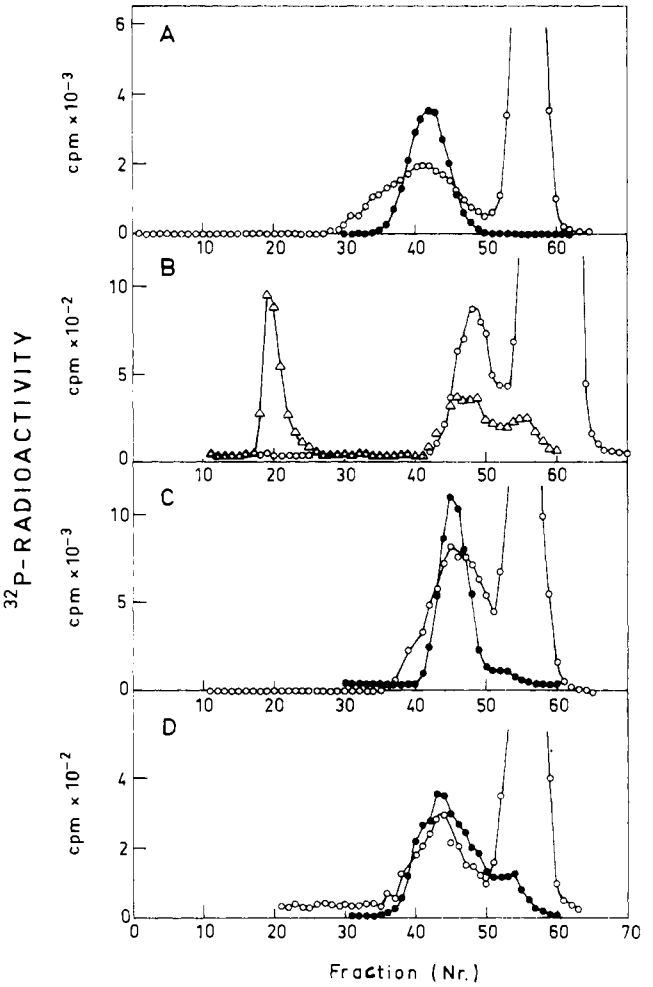

FIGURE 3: Fractionation of ligase reaction products on a combination column of Sephadex G75 and Bio-Gel A 1.5 m. A 50-μl sample of the reaction mixture was treated as described in the Experimental Section. (A) Product of joining reaction of oligo(rU) on poly(rA) (○). The elution profile of oligo(rU) is also shown (●). (B) Product of joining reaction of oligo(rA) on poly(rU) (○) and of oligo(rA) on poly(dT) (△). (C) Product of joining reaction of oligo(rI) on poly(rC) (○). The elution profile of oligo(rI) is also shown (●). (D) Product of joining reaction of oligo(rC) on poly(rI) (○). The elution profile of oligo(rC) is also shown (●).

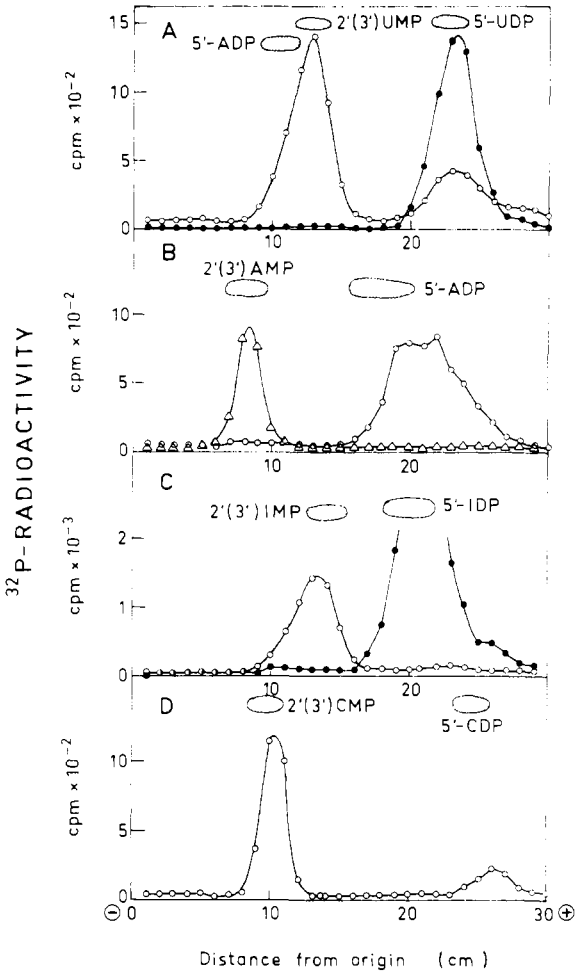

FIGURE 4: High-voltage paper electrophoresis of products formed by total alkaline hydrolysis of ligase-joined oligonucleotides. (A) Product from poly(rA)-oligo(rU) reaction (○). For control, the chromatographic position of the mononucleotide diphosphate is shown by the radioactivity profile of [5'-^{32}P]-3'(2')-uridine diphosphate obtained by alkaline hydrolysis of the oligo(rU) (●). (B) Products from poly(rU)-oligo(rA) reaction (○) and from poly(dT)-oligo(rA) reaction (△). (C) Product from poly(rC)-oligo(rI) reaction (○); control of 5'(2'),3'-IDP (●). (D) Product from poly(rI)-oligo(rC) reaction (○). The shaded areas indicate the positions of marker nucleotides identified by ultraviolet-light quenching.

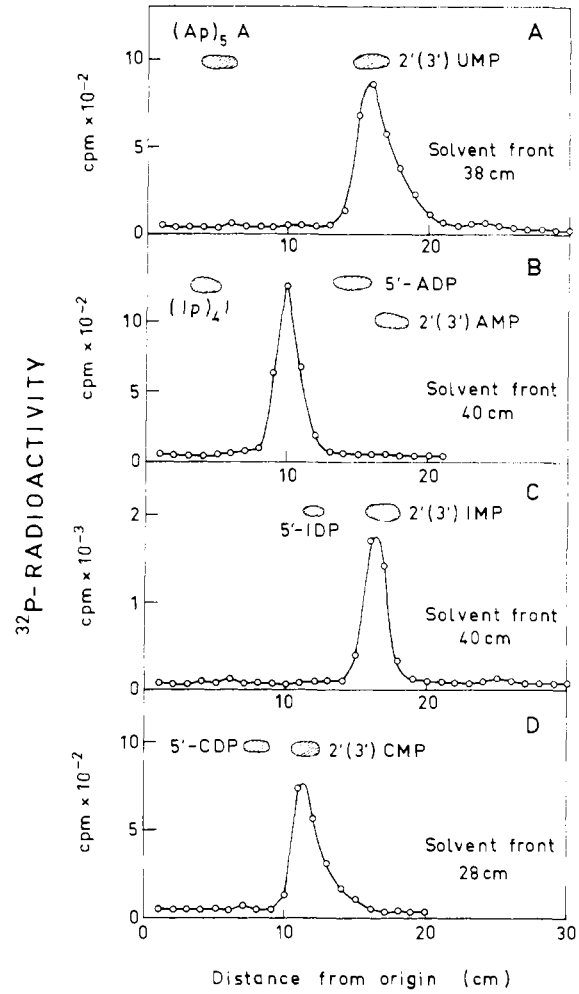

FIGURE 5: Paper chromatography of total alkaline hydrolyzed products eluted with 0.25% ammonia solution from papers subjected to high voltage electrophoresis (Figure 4). The material was analyzed in the 1-propanol solvent system described in the Experimental Section: (A) product from poly(rA)-oligo(rU) reaction; (B) product from poly(rU)-oligo(rA) reaction; (C) product from poly(rC)-oligo(rI) reaction; (D) product from poly(rI)-oligo(rC) reaction. The shaded areas indicate the position of marker nucleotides identified by ultraviolet-light quenching.

markers. The ^{32}P-labeled material corresponding to the nucleotide monophosphate position was eluted from the paper and after concentrating analyzed further by descending paper chromatography. Figure 5 shows that again the ^{32}P radioactivity peak coincided with the nucleotide monophosphate position. These results prove that DNA ligase had indeed covalently connected oligonucleotides by the formation of phosphodiester bonds. In the case of oligo(rA), however, no ^{32}P-labeled adenosine monophosphate could be detected with these techniques, indicating that oligo(rA) cannot be sealed on a poly(rU) template under the conditions used. This agrees with the recent finding of Kleppe *et al.* (1970) who were unable to ligate oligo(rA) on poly(rU) with DNA ligase. Similar to these authors, we found that oligo(rA) does form an alkaline phosphatase resistant complex which moves slightly faster than ADP in the electrophoresis (Figure 4B) and rather slowly in the 1-propanol system (Figure 5B). We have not further analyzed the nature of this product.

It is worth mentioning that DNA ligase will work on a substrate consisting of oligo(rU) and oligo(rA) present in equal amounts by connecting only oligo(rU) molecules with each other and leading again to the same alkaline phosphatase resistant complex of oligo(rA) in which no internucleotide bond has been formed (data not shown).

For an estimation of the extent of the RNA ligase activity of DNA ligase in comparison to the joining activity on an efficient substrate such as poly(dT)-oligo(rA), various 5'-^{32}P-labeled substrate combinations were treated with DNA ligase under the conditions of this study. The obtained amounts of alkaline phosphatase resistant radioactivities are listed in Table I. In the experiments which were carried through the paper electrophoresis step, the amounts of ^{32}P radioactivities found in the nucleotide monophosphate position are indicated. It can be seen that, after 20 hr of incubation, from 9 to 38% of the input oligoribonucleotides had become alkaline phosphatase resistant. These values are also indicative of the degree of phosphodiester bonds formed, since almost all the radioactivities could be recovered as the corresponding nucleotide monophosphates after alkaline hydrolysis, as shown in the table. In the case of oli-

go(rA), however, no internucleotide linkage was formed, as discussed above.

Discussion

As evident from the kinetic analysis of the enzymatic reaction and from the transfer of ^{32}P radioactivity from the 5′ end of substrate oligoribonucleotides to the 3′ end of nucleotide monophosphates after a total alkaline hydrolysis of the reaction product, it is clear that, under the incubation conditions used, DNA ligase of phage T4 behaves as an RNA ligase. A head-to-tail joining of oligo(rU), oligo(rI), and oligo(rC), respectively, was observed in the presence of equal amounts of the corresponding complementary polyribonucleotides. This RNA ligase activity tested under the DNA ligase assay conditions needs enzyme concentrations higher than those used for sealing of nicked DNA but comparable to those used for joining of oligonucleotides in DNA–RNA hybrid structures (Kleppe *et al.*, 1970). The RNA ligase gives rise to rather short products only, but we feel that the efficiency of this RNA ligase activity may be improved by optimizing the conditions. The main purpose of the present study was to show the basic feature of this new reaction catalyzed by DNA ligase. Oligo(rA), however, neither in combination with poly(rU) nor with oligo(rU) is a substrate for the ligase under the conditions used. The reason for this failure of head-to-tail joining of oligo(rA) with RNA ligase activity of DNA ligase is unknown and may lie in special structural features of the oligo(rA)–poly(rU) complex.

The RNA ligase activity described in this paper is exercised by a DNA ligase sample prepared by the established method and exhibiting normal characteristics of DNA ligase such as activity on nicked DNA and a K_m value for ATP of 1.0×10^{-5}M (Weiss *et al.*, 1968) (data not shown). Whether the RNA and DNA ligase activities of the preparation are catalyzed by the same protein or whether the phosphocellulose chromatography fraction used in this work contained more than one polynucleotide ligase is unknown at present. The enzyme isolated differs, however, from the recently described RNA ligase of T4 phage infected cells. According to Linné *et al.* (1974), this RNA ligase is eluted from DEAE-cellulose only at much higher salt molarities and is free of DNA ligase activity. This enzyme was assayed with high molecular weight synthetic RNAs such as poly(rI)–poly(rC) and poly(rU)–poly(rA). Another RNA ligase preparation, isolated from T4 infected *E. coli* by Silber *et al.* (1972), was shown to act preferentially on poly(rA) circularizing this substrate.

An *in vivo* function of any of these RNA ligase activities in T4-infected *E. coli* cells is not obvious at the moment. But, for synthetic purposes, the RNA ligase activity of DNA ligase, as well as the other RNA ligases, will certainly be of great value.

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