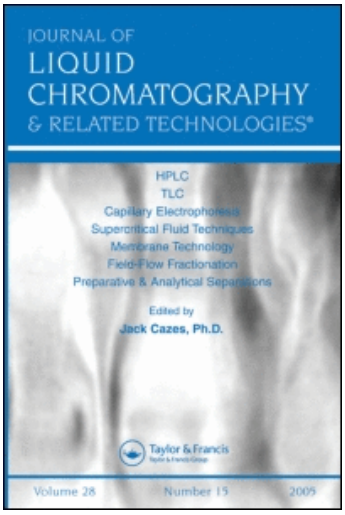

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ENZYMATIC SYNTHESIS OF AN RNA FRAGMENT CORRESPONDING TO THE ANTICODON LOOP AND STEM OF $tRNA^{PHE}$ FROM YEAST

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ABSTRACT

A hexadecamer corresponding to the anticodon loop and stem of $tRNA^{Phe}$ yeast has been prepared using T_4 RNA ligase and isolated by high performance liquid chromatography. The two oligonucleotides used in the ligation were isolated from a ribonuclease T_1 digest of the tRNA which was resolved by HPLC on an anion exchange column. To prepare the "acceptor" oligonucleotide for the RNA ligase reaction a 3' terminal phosphate was removed. To prepare the "donor" oligomer a 5' terminal phosphate was added. Analysis of the product hexadecamer was by nucleoside and nucleotide-3'-monophosphate composition.

INTRODUCTION

RNA fragments of defined sequence can be useful in the study of the mechanisms involved in protein synthesis. Such fragments can be prepared chemically, enzymatically or by a combination of both techniques. Chemical oligoribonucleotide synthesis, while very efficient for short oligomers, results in poor yields as the length of the oligomer increases. The chemical synthesis of short RNA pieces followed by combining these fragments with T_4 RNA ligase to produce longer fragments appears at present to be the most efficient approach.

Chemical synthesis of oligomers is also of less value when modified nucleotides are necessary in the sequence owing to the difficulty or expense of obtaining enough material. It is sometimes simpler to isolate oligonucleotides containing the desired modified nucleotides from a naturally occurring RNA and then link them together in the desired sequence using RNA ligase. T_4 RNA ligase joins together a "donor" oligonucleotide containing a 5' phosphate with an "acceptor" oligonucleotide containing an unsubstituted 3' hydroxyl in a three step mechanism (1,2) during which ATP is converted to AMP. The reaction yields are not drastically affected by oligomer length although the efficiency of the reaction does have some base sequence dependence (3,4). Additionally, the enzyme will tolerate a large number of modified nucleotides.

We wish to report the synthesis of a hexadecamer corresponding to the anticodon loop and stem of yeast $tRNA^{Phe}$. The oligonucleotides necessary to prepare this fragment were excised from the native tRNA with a ribonuclease and resolved by high performance liquid chromatography (HPLC). The isolated oligonucleotides were then modified such that they contained either a 3' hydroxyl or 5' phosphate and then ligated together using T_4 RNA ligase.

MATERIALS

Potassium dihydrogen phosphate (Merck, Darmstadt, FRG) and analytical grade methanol (J.T. Baker, Phillipsburg, Pa. USA) were commercial preparations and used as purchased. Adenosine (A), guanosine (G), cytidine (C), uridine (U) and their respective 3'-monophosphates (Ap, Gp, Cp, Up) and ATP were from Sigma Chemical Co. (Munich, FRG). [γ-^{32}P]ATP was a product of Amersham Buchler (Braunschweig, FRG). Pseudouridine (Ψ), its 3'-monophosphate (Ψp), 5-methylcytidine (m^5C), 2'-O-methylcytidine (Cm), 2'-O-methylguanosine (Gm), bacterial alkaline phosphatase (E.C. 3.1.3.1.) and snake venom phosphodiesterase (E.C. 3.1.4.1.) were from Boehringer (Mannheim, FRG). Polynucleotide kinase (E.C. 2.7.1.78) (lacking the 3'-phosphatase activity) was purchased from New England Nuclear

(Dreieich, FRG). RNase T_1 and RNase T_2 were from Sankyo through Koch-Light Lab. (Frankfurt, FRG). Transfer RNA specific for phenylalanine ($tRNA^{Phe}$) was isolated according to published procedures (5). T_4 RNA ligase (E.C. 6.5.1.3.) was prepared by a modification of published procedures and will be described elsewhere.

EQUIPMENT

Oligoribonucleotide separations were performed on a Dupont 850 LC liquid chromatograph (Dupont, Bad Nauheim, FRG) equipped with a Hewlett Packard 3380 A integrator. 4.6 x 250 mm columns of APS-Hypersil or ODS-Hypersil (Shandon Southern, Ltd., Runcorn, England) were packed as described previously (6,7). 4.6 x 250 mm and 9.4 x 250 mm columns of Zorbax-NH_2 were from Dupont.

The nucleoside/nucleotide-3'-monophosphate separation was performed on an HPLC system assembled in this laboratory consisting of a Milton Roy pump (Dosapro Milton Roy, Neu-Isenburg, FRG), a 4.6 x 250 mm ODS-Hypersil column, a Dupont 840 260 nm detector and suitable recorder.

METHODS

Ribonuclease T_1 Hydrolysis of $tRNA^{Phe}$ Yeast

To 50 A_{260} units $tRNA^{Phe}$ yeast in 100 µl 50 mM Tris-HCl pH 7.0 was added 250 units RNase T_1. After a 4 h incubation the mixture was chromatographed on a 4.6 x 250 mm APS-Hypersil column at 35°C with a flow rate of 2 ml/min. Buffer A: 0.05 M KH_2PO_4 pH 4.5, buffer B: 10 % CH_3OH in 0.9 M KH_2PO_4 pH 4.5. Gradient: 0-60 min, 0-100 % buffer B; 60-80 min, 100 % buffer B.

To 500 A_{260} units $tRNA^{Phe}$ yeast in 0.5 ml 50 mM Tris-HCl pH 7.0 was added 1500 units RNase T_1. After overnight incubation at 37°C the mixture was chromatographed in 3 aliquots on a 9.4 x 250 mm Zorbax-NH_2 column at a flow rate of 5 ml/min with conditions as described above.

Preparation of CpCpApG

To 2.1 µmol of CpCpApGp (50 A_{260} units) in 0.6 ml 100 mM ammonium acetate pH 8.8 and 10 mM $MgCl_2$ was added 20 µl bacterial alkaline phosphatase (1 mg/ml). After a 60 min incubation at 37°C, HPLC analysis indicated the reaction was complete. The reaction mixture was adsorbed on a 5 ml Sephadex A-25 column. The enzyme was eluted with 0.02 M sodium acetate pH 5.2 containing 0.2 M NaCl and the oligomer eluted with 0.02 M sodium acetate pH 5.2 containing 0.8 M NaCl. After desalting of the oligomer using a 1.5 x 50 cm Sephadex G-10 column 1.9 µmol (46 A_{260} units) of CpCpApG was obtained. Yield: 92 %.

Preparation of pApCmpUpGmpApApYpApΨpm^{5}CpUpGp

To 0.5 µmol (35 A_{260} units) of the dodecamer isolated from the RNase T_1 digestion of $tRNA^{Phe}$ yeast in 50 mM Tris-HCl pH 9.5, 10 mM $MgCl_2$, 10 mM mercaptoethanol and 50 µg/ml bovin serum albumin containing 2 mM ATP including varying amounts of [γ-^{32}P]ATP was added 150 units polynucleotide kinase. After a 60 min incubation at 37°C, the reaction mixture was adsorbed on a 5 ml Sephadex A-25 column. A step gradient as described for the preparation of CpCpApG was employed which after desalting resulted in 0.42 µmol (30 A_{260} units) of the 5' phosphorylated dodecamer. Yield: 84 %.

Preparation of CpCpApGpApCmpUpGmpApApYpApΨpm^{5}CpUpGp

To 1.0 µmol (25 A_{260} units) CpCpApG and 0.34 µmol (25 A_{260} units) of pApCmpUpGmpApApYpApΨpm^{5}CpUpGp in 1.0 ml 50 mM HEPES pH 8.4, 20 mM $MgCl_2$, 3.3 mM dithioerythritol, 3 mM ATP and 10 µg/ml bovin serum albumin was added 230 units T_4 RNA ligase. After an 18 h incubation at 17°C the product was isolated by chromatography on ODS-Hypersil using buffer A: 0.02 M KH_2PO_4 pH 5.5, buffer B: 70 % CH_3OH in 0.02 M KH_2PO_4 pH 5.5 and a 60 min gradient from 0-50 % buffer B. The isolated oligomer was desalted on a 1.5 x 50 cm Sephadex G-10 column which resulted in 0.17 µmol (16 A_{260} units) of the desired hexadecamer. Yield: 50 %.

Nucleoside Analysis

To 0.4-1.0 A_{260} unit of oligoribonucleotide in 40 µl 50 mM ammonium acetate pH 8.8 and 10 mM $MgCl_2$ was added 3 µl snake venom phosphodiesterase (1 mg/ml) and 3 µl bacterial alkaline phosphatase (1 mg/ml). After an 18 h incubation at 37°C the resultant nucleoside mixture was analyzed by HPLC on an ODS-Hypersil column.

Nucleoside/Nucleotide-3'-Monophosphate Analysis

To 0.5 A_{260} unit of oligoribonucleotide in 50 µl 50 mM sodium acetate pH 5.0 was added 5 units RNase T_2. After a 2 h incubation at 37°C the resultant mixture was analyzed by HPLC on an ODS-Hypersil column.

The following extinction coefficients have been used at pH 5.5: Cytidine and Cp, 8.58×10^3; uridine and Up, 10.1×10^3; guanosine and Gp, 11.5×10^3; adenosine and Ap, 13.6×10^3; pseudouridine and Ψp, 9.98×10^3; 5-methylcytidine, 6.06×10^3; 2'-O-methylcytidine, 8.41×10^3 and 2'-O-methylguanosine, 11.31×10^3.

RESULTS

An RNA fragment corresponding to the anticodon loop and stem of $tRNA^{Phe}$ was prepared by joining together two oligoribonucleotides which have been isolated from an RNAse T_1 hydrolysis of the tRNA. Although some modified guanosine residues are resistant to hydrolysis, ribonuclease T_1 is very specific for hydrolysis at guanosine. In the case of $tRNA^{Phe}$ from yeast the hydrolysis results in 15 unique oligonucleotides and guanosine-3'-monophosphate. A chromatogram showing the resolution of approximately 2.5 mg of this mixture on a 4.6 x 250 mm Zorbax-NH_2 column is shown in Figure 1. While some of the dinucleoside diphosphates are not clearly separated, the longer oligonucleotides are well resolved. This digestion can be scaled up to 500 A_{260} units (25 mg) and isolated on a 9.4 x 250 mm Zorbax-NH_2 column. The resolution is very similar to that shown in Figure 1 although the oligonucleotides CpUpCpApGp

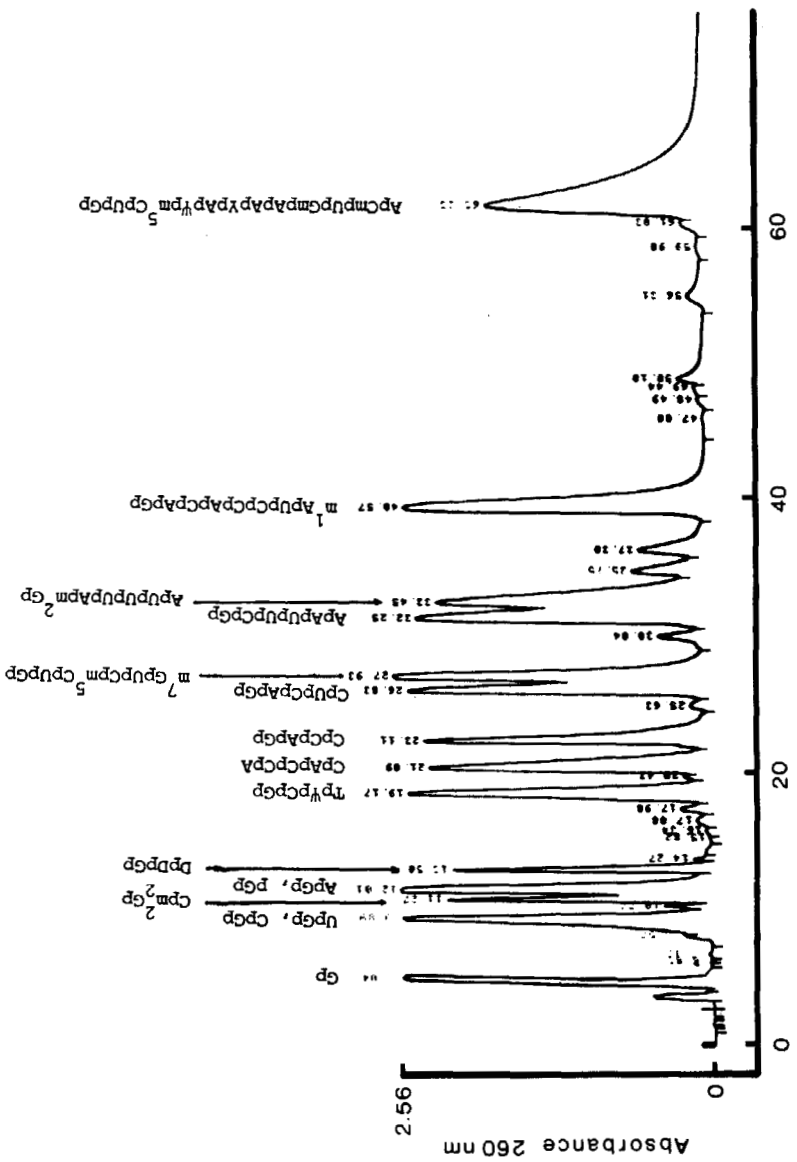


FIGURE 1. Chromatography of 50 A_{260} units of a ribonuclease T_1 hydrolysate of $tRNA^{Phe}$ yeast. Column: 4.6 x 250 mm Zorbax-NH_2, Temperature: 35°C, Flow: 2 ml/min, Detector: 260 nm, 2.56 aufs. Buffer A: 0.05 M KH_2PO_4 pH 4.5. Buffer B: 10% CH_3OH in 0.9 M KH_2PO_4 pH 4.5. Gradient: 0-60 min, 0-100% buffer B; 60-80 min, 100% buffer B.

and $m^7GpUpCpm^5CpUpGp$ as well as ApApUpUpCpGp and $ApUpUpUpApm^2Gp$ co-elute. However, the two oligonucleotides necessary for the present synthesis, CpCpApGp eluting at 23 min (Fig. 1) and $ApCmpUpGmpApApYpAp\Psi pm^5CpUpGp$ eluting at 63 min (Fig. 1) remain well resolved and were easily isolated.

RNase T_1 produces oligonucleotides with 3' terminal phosphates and 5' terminal hydroxyl groups and T_4 RNA ligase requires a 3' terminal hydroxyl and a 5' terminal phosphate. Therefore, the acceptor molecule for the present synthesis must have its 3' terminal phosphate removed and a 5' terminal phosphate must be added to the donor molecule.

The 3' phosphate of CpCpApGp was removed using bacterial alkaline phosphatase and the extent of reaction monitored by HPLC using the APS-Hypersil anion exchange column. The chromatogram of Figure 2 shows the analysis of the reaction mixture just prior to the addition of the phosphatase and after a 60 min incubation. The analysis at 60 min indicated the reaction had gone to completion and no sig-

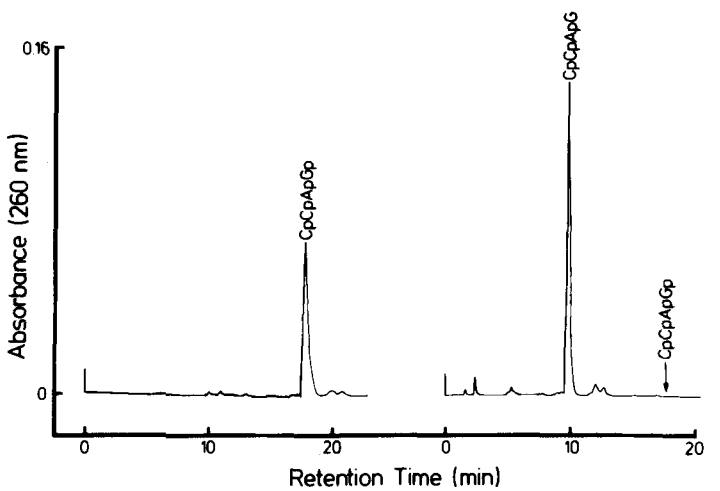


FIGURE 2. Analysis of the dephosphorylation of CpCpApGp to produce CpCpApG. Conditions as described in Fig. 1 with following exceptions: Column: 4.6 x 250 mm APS-Hypersil, Detector: 260 nm, 0.16 aufs.

nificant oligomer degradation had occurred. While HPLC can be used to isolate the product oligomer from this reaction it is not necessary since only one oligonucleotide is present in the solution. In this case it was easier to adsorb the reaction mixture on a soft gel anion exchanger, elute the enzyme from the column and then the oligonucleotide. HPLC analysis after desalting and concentration of the product indicated a single peak.

The 5' terminal phosphate necessary for the donor molecule in the RNA ligase reaction was added using polynucleotide kinase. The donor molecule, in this case the dodecamer, should preferentially have a blocked 3' terminal hydroxyl to prevent self ligation of two donor molecules or ligation of a second donor molecule onto the product resulting from one acceptor plus one donor oligonucleotide. A 3' terminal phosphate will act as a blocking group although this can result in some side reactions (8). However, it is difficult to transfer a phosphate group to the 3' terminal hydroxyl. One advantage of using RNase produced oligoribonucleotides is that they have already the 3' terminal hydroxyl blocked with a phosphate group.

The chromatograms showing the analysis on an ODS-Hypersil column of the dodecamer prior to phosphorylation with ATP and polynucleotide kinase as well as the analysis after a 60 min incubation with the enzyme are reproduced in Figure 3. Since the HPLC analysis indicated only one oligonucleotide product, a small Sephadex A-25 column was again used for its isolation.

The acceptor molecule, CpCpApG, and the donor molecule, pApCmpUpGmpApApYpApΨpm^{5}CpUpGp, were then joined together using RNA ligase. A chromatogram of the analysis of the ligation reaction mixture by HPLC using an ODS-Hypersil column is shown in Fig. 4. The major oligonucleotide product was observed to elute at 26 min in the gradient described in Figure 4 as monitored by both UV absorbance and scintillation counting. The product was isolated using a 4.6 x 250 mm ODS-Hypersil column and the gradient conditions described in Figure 4. The pH of the solution was adjusted such that

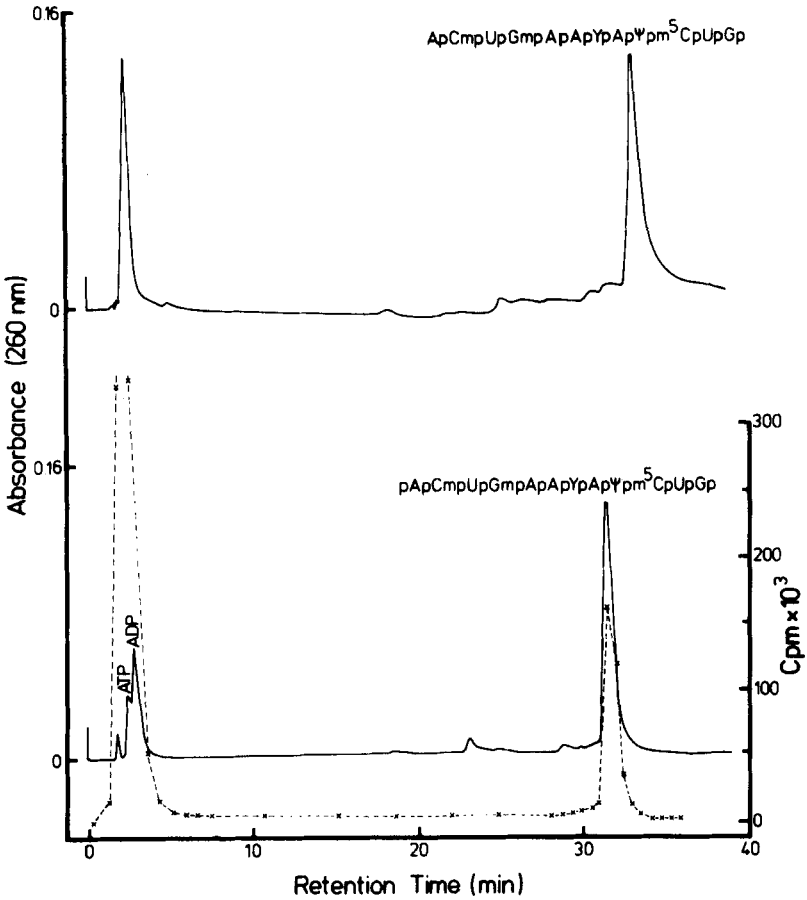


FIGURE 3. Analysis of the dodecamer phosphorylation. Column: 4.6 x 250 mm ODS-Hypersil, Temperature: 35°C, Flow: 1.5 ml/min, Detector: 260 nm, 0.16 aufs. Buffer A: 0.02 M KH_2PO_4 pH 5.5. Buffer B: 70 % CH_3OH in 0.02 M KH_2PO_4 pH 5.5. Gradient: 0-60 min, 0-50 % buffer B.

it was below 7 prior to chromatography by three injections of the reaction mixture.

After desalting and concentration of the product it was analyzed for nucleoside composition as shown in Figure 5. The standard chromatogram of Figure 5a shows the retention times for the nucleo-

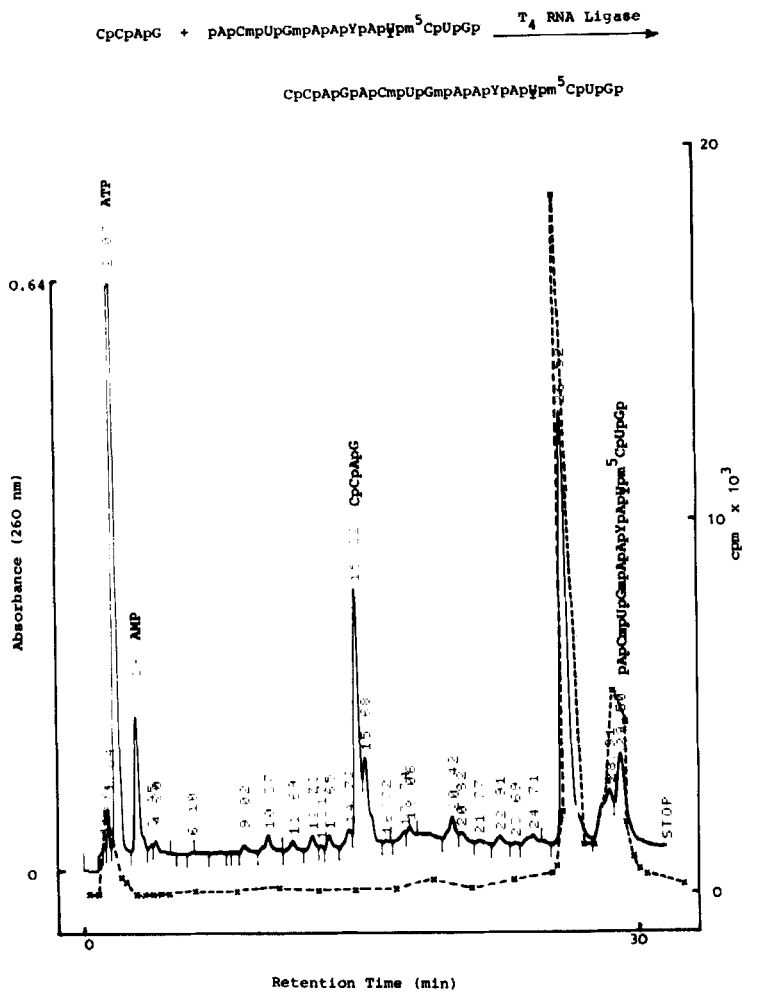


FIGURE 4. Analysis of the ligation of the tetramer from Fig. 2 to the dodecamer from Fig. 3. Column and conditions as described in Fig. 3.

sides present in the hexadecamer, excluding the Y base (Wybutosine) which is not eluted under these conditions. The chromatogram of Figure 5b shows the nucleoside analysis of the dodecamer isolated from the yeast $tRNA^{Phe}$. The nucleoside analysis of the product oligomer is shown in Figure 5c. It is clear from the latter analy-

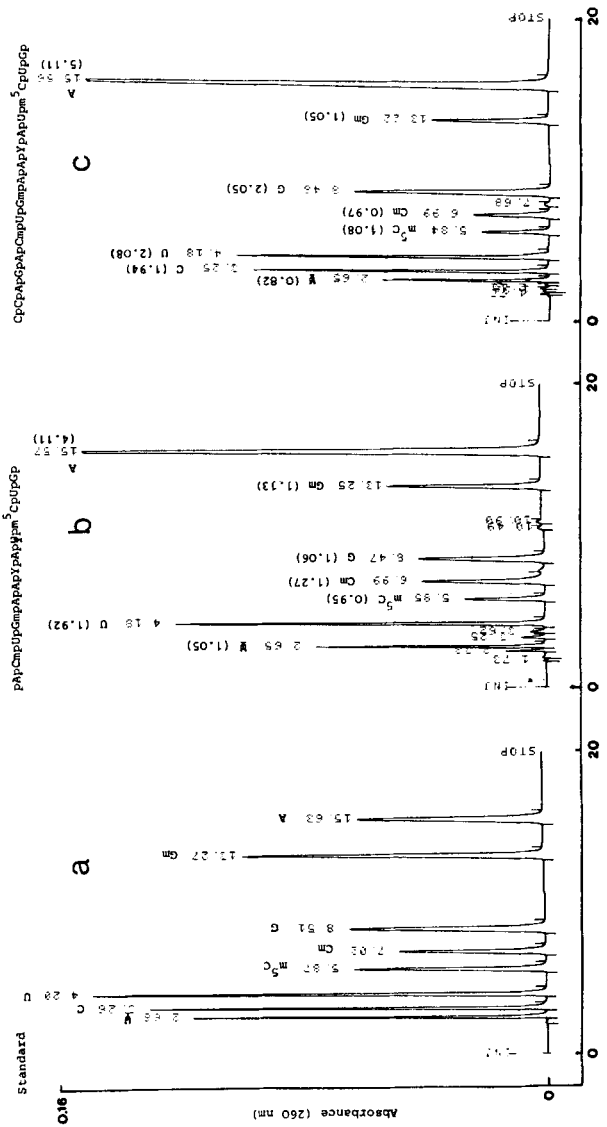


FIGURE 5. Nucleoside analysis of oligonucleotides using snake venom phosphodiesterase and bacterial alkaline phosphatase a) A standard nucleoside mixture. b) Analysis of the starting "donor" dodecamer. c) Analysis of the product hexadecamer. Column: 4.6 x 250 mm ODS-Hypersil, Temperature: 35°C, Flow: 1.5 ml/min, Detector: 260 nm, 0.16 aufs. Buffer A: 2.5 % methanol in 0.01 M $NH_4H_2PO_4$ pH 5.5. Buffer B: 8 % methanol in 0.01 M $NH_4H_2PO_4$ pH 5.5. Gradient: 0-5 min, 0 % buffer B; 5-20 min, 100 % buffer B.

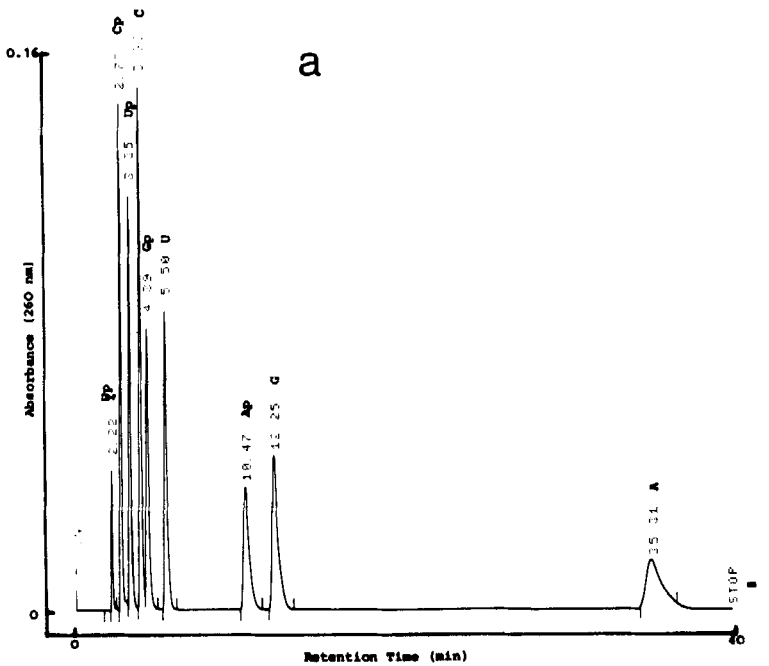


FIGURE 6. Nucleoside-Nucleotide analysis using RNase T_2. a) A standard mixture of the four common nucleosides and their respective 3'-monophosphates. b) Analysis of the product hexadecamer. Column: 4.6 x 250 mm ODS-Hypersil, Temperature: 35°C, Flow: 1.5 ml/min, Detector: 260 nm, 0.16 aufs. Isocratic elution using 0.02 M KH_2PO_4 pH 5.5 containing 1 % methanol.

sis that in addition to the nucleosides present in the donor molecule, two equivalents of cytidine, and one additional equivalent of both guanosine and adenosine appear in the hexadecamer product.

In addition to nucleoside analysis, nucleoside-3'-monophosphate analysis will also yield useful information regarding the product. The 5' terminal phosphate of the donor molecule in the RNA ligase reaction is additionally bound to the 3' terminal nucleoside of the acceptor molecule. After RNase T_2 (a non-specific ribonuclease) digestion of the product oligomer, the nucleoside-3'-

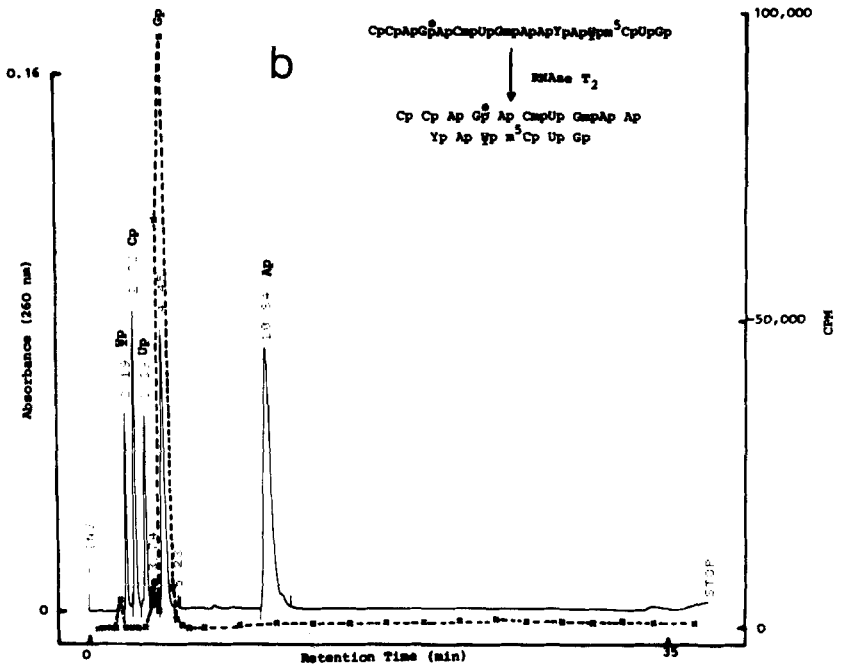


FIGURE 6B

-monophosphate resulting from the 3' terminal nucleotide of the acceptor molecule now bears this phosphate. By using isotopically labelled phosphate it is possible to monitor that the 5' terminal phosphate of the donor is bound to the 3' terminal nucleoside of the acceptor.

The separation of a standard mixture of nucleosides and nucleoside-3'-monophosphates is shown in Figure 6a. The RNase T_2 analysis of the hexadecamer is shown in Figure 6b. While some of the modified nucleoside-3'-monophosphates as well as the two dinucleoside diphosphates produced are not eluted under the described isocratic conditions, it is clear that the radioactivity is associated with the guanosine-3'-monophosphate peak.

DISCUSSION

Since oligonucleotides are polyanions containing lipophilic bases both anion-exchange and reverse-phase chromatography are potentially useful for separation and purification. For complex mixtures of oligonucleotides such as that produced from the RNase T_1 digestion of Figure 1, anion-exchange chromatography is often preferred as a first step. In the chromatography of this mixture of oligonucleotides at pH 4.5 the dodecamer binds very strongly to the column. It was observed that adding a small amount of methanol to buffer B assisted in the elution of the dodecamer from the column. This may reflect lipophilic interactions between the solute and stationary phase most probably involving the Y base. Resolution of the mixture occurs largely according the chain length with the longer fragments eluting later. In cases where resolution by anion exchange is not sufficient that pure oligonucleotides can be collected a second step involving reverse-phase chromatography can be used. For example the oligomers CpUpCpApGp and m^7GpUpCpm^5CpUpGp as well as ApApUpUpCpGp and ApUpUpUpApm^2Gp which are not completely resolved on the Zorbax-NH_2 anion-exchange column (Fig. 1) were collected together. Subsequent chromatography on an ODS reverse-phase column resulted in well separated peaks for either of the two pairs of oligonucleotides.

The anion-exchange column we presently favor contains an aminopropylsilyl (APS) bonded phase support. In contrast to strong anion exchange (SAX) supports which contain tetraalkylammonium salts, the APS support is an anion-exchanger only in the presence of acidic buffers. Therefore, the number of cationic sites available to bind an anionic solute is pH dependent. At low pH oligonucleotides will be bound much more strongly than near neutral pH. It is therefore possible to run salt gradients at different pHs or pH gradients at different salt concentrations with this stationary phase.

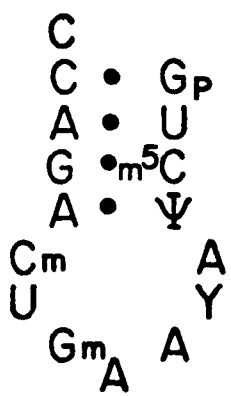
Oligonucleotides of lengths 1-10 are generally well resolved on the APS support at pH 4.5. The addition or loss of a phosphate

group can be easily monitored as illustrated for the tetramer in Figure 2. While it was impossible to analyze the phosphorylation of the dodecamer under these conditions, using the same gradient conditions at pH 6.5 both the 5' phosphorylated and 5' hydroxy dodecamer could be resolved with relatively short retention times.

The octadecasilyl (ODS) stationary phase can also be used with success to analyze phosphorylation or dephosphorylation reactions. The addition of a 5' terminal phosphate to the dodecamer of Figure 3 results in a product monitored both by UV absorbance and scintillation counting, with a shorter retention time than the starting material. Since chromatography by reverse-phase relies upon lipophilic interactions with the stationary phase, by increasing the polarity of the solute (e.g. addition of a phosphate group) a decrease in retention time is observed.

Reverse-phase chromatography of oligonucleotides does not necessarily result in elution according to polymer length. Since lipophilic interactions are involved and purine residues are more strongly retained than pyrimidine residues, a shorter oligonucleotide with a high purine content may have a longer retention time than a longer oligomer composed largely of pyrimidines.

Secondary structure may also influence migration velocity along the ODS support. For example, the hexadecamer of Figure 4 elutes earlier than the starting dodecamer. Since the hexadecamer corresponds to the anticodon loop and stem of $tRNA^{Phe}$ yeast the following secondary structure is possible:



While it cannot be determined if the oligonucleotide chromatographs with a base paired structure, it can be assumed that if it were so the lipophilic bases would be turned toward the center of the helix and the phosphate groups to the outside. The bases would be less able to interact with the stationary phase and the retention time would be shorter than otherwise expected.

A simple nucleoside analysis of a product oligonucleotide using snake venom phosphodiesterase and bacterial alkaline phosphatase is of particular value if modified nucleosides are present. In cases where only the four common nucleosides are present an analysis which produces nucleotides and/or nucleosides is often of more value. Separation of a standard nucleoside and nucleoside-3'-monophosphate mixture is shown in Figure 6a. While the retention times, particularly of adenosine can be reduced by the use of a methanol gradient, the isocratic elution described here allows the use of a very simple HPLC system (see METHODS).

The analysis described here can also yield information concerning the terminal nucleoside. If no 3' terminal phosphate is present, the RNase T_2 digestion of an oligonucleotide will produce nucleoside-3'-monophosphates except for the 3' terminus which is released as a nucleoside. An analogous digestion and analysis can be done using snake venom phosphodiesterase which will produce nucleoside-5'-phosphates. The RNase T_2 analysis is however useful when the oligonucleotide has been prepared using RNA ligase. It is then possible to observe that the terminal phosphate of the donor oligonucleotide elutes as the nucleoside-3'-monophosphate indicating the 3' terminal nucleoside of the acceptor oligonucleotide (Fig. 6b).

The biological effects of this hexadecamer in protein synthesis is presently under study.

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REFERENCES

1. Gumport, R.I. and Uhlenbeck, O.C., Gene Amplification and Analysis. Analysis of Nucleic Acid Structure by Enzymatic Methods, Chirikjian, I.G. and Papas, T.S., eds., Elsevier/North Holland, New York, 1981.
2. Uhlenbeck, O.C. and Gumport, R.I., The Enzymes, 3rd ed., Vol. 14 in press.
3. England, T.E. and Uhlenbeck, O.C., Enzymatic Oligoribonucleotide Synthesis with T_4 RNA ligase, Biochemistry 17, 2069, 1978.
4. Romaniuk, E., McLaughlin, L.W., Neilson, T. and Romaniuk, P.J., The Effect of Oligoribonucleotide Sequence on the T_4 RNA Ligase Reaction, Eur. J. Biochem., in press 1982.
5. Schneider, D., Solfert, R. and von der Haar, F., Large Scale Purification of $tRNA^{Ser}$, $tRNA^{Tyr}$ and $tRNA^{Phe}$ from Baker's Yeast, Hoppe-Seyler's Z. Physiol. Chem. 353, 1330, 1972.
6. McLaughlin, L.W., Cramer, F. and Sprinzl, M., Rapid Analysis of Modified $tRNA^{Phe}$ from Yeast by High Performance Liquid Chromatography: Chromatography of Oligonucleotides after RNase T_1 Digestion on Aminopropylsilica and Assignment of the Fragments based on Nucleoside Analysis by Chromatography on C-18 silica, Anal. Biochem. 112, 60, 1981.
7. McLaughlin, L.W. and Romaniuk, E., The Use of High Performance Liquid Chromatography in the Isolation and Analysis of Oligoribonucleotides synthesized by the T_4 RNA Ligase Reaction, Anal. Biochem., in press 1982.
8. Krug, M. and Uhlenbeck, O.C., Reversal of T_4 RNA Ligase, Biochemistry 21, 1858, 1982.