BBA 95658

SYNTHESIS OF POLYSPONGOURIDYLIC ACIDS FROM URIDYLIC ACIDS AND PHENYLPOLYPHOSPHATE ESTERS

G. SCHRAMM UND I. ULMER-SCHÜRNBRAND
Max-Planck-Institut für Virusforschung, Tübingen (Germany)
(Received December 29th, 1966)

SUMMARY

Condensation of uridylic acid with phenylpolyphosphate ester leads to a new type of polynucleotide. The intermediary formation of poly-O^2-2'-cyclouridylates and their subsequent hydrolysis leads to inversion of the ribose 2'-hydroxyl group to produce polyarabino nucleotides. These are resistant to ribonuclease and show a high stability in alkali and acid. This stability permits the removal of polyuridylic acid. The acid-stable condensation products can be separated chromatographically and oligonucleotides with $n = 2$ to $n > 10$ are obtained. Degradation with bacterial monophosphatase and snake-venom diesterase yields 9-β-D-arabinosyl uracil (spongouridine). It is concluded that the polyspongouridylic acids have 3'-5' phosphate-diester linkages. Polyspongouridylates have a high susceptibility to pancreatic ribonuclease and inhibit ribonuclease digestion of RNA. They stimulate the adaptation of phenylalanine-tRNA to ribosomes.

INTRODUCTION

SCHRAMM, GRÖTSCH AND POLLMANN[1] prepared ethylpolyphosphate esters by reaction of P_4O_{10} with diethyl ether using the method of K. LANGHELD[2,3]. By heating nucleotides with this ethylpolyphosphate ester, polynucleotides were formed differing in certain properties from the natural nucleic acids. The products obtained by condensation of ribonucleotides were partially stable to alkaline hydrolysis, whereas the natural polyribonucleotides are easily hydrolyzed in alkaline solution. These differences have also been observed by other authors[4-6]. In order to clarify the mechanism of the polynucleotide formation, it was necessary to study the structure and reactivity of ethylpolyphosphate esters in more detail. It was found that ethylpolyphosphate ester not only has the desired phosphorylating effect, but also leads to alkylation[7]. This disturbing side reaction would not be expected with phenyl-

Abbreviation: Su, spongouridine (9-β-D-arabinosyluracil).

polyphosphate esters and an attempt was therefore made to prepare phenylpolyphosphate ester by reaction of P_4O_{10} and diphenyl ether. These experiments were not successful, but phenylpolyphosphate ester preparations of various compositions could be obtained with P_4O_{10} and triphenyl phosphate under conditions which lead to reorganization. The structures of the resulting products were elucidated by nuclear magnetic resonance[8]. The preparation of phenylpolyphosphate ester is simple and easily reproducible. These esters proved very useful for the preparation of polyamino acids, polysaccharides, purine nucleosides, and for the condensation of different deoxyribo- and ribonucleotides. In this paper only those experiments dealing with the condensation of uridylic acid by phenylpolyphosphate ester will be described.

We obtained polyarabinonucleotides from uridylic acid by inversion of the hydroxy group at C-2'. Some of the chemical and biological properties of this new type of polynucleotide will be reported.

METHODS

Preparation of phenylpolyphosphate ester

A mixture of 44 g dry P_4O_{10} (0.16 mole) and 130 g dry triphenylphosphate ester (0.4 mole) was heated to 300–320° with continuous stirring. After about 1 h a clear solution was obtained, which was very viscous at room temperature, but which could be handled easily at 50°.

Preparation of polyspongouridylic acids

1 g of disodium uridine (2')3'-phosphate (24 000 absorbance units) (Boehringer, Mannheim), which had been dried 1 h at 105°, and 10 g phenylpolyphosphate ester were mixed and heated at 100° for 24 h in a closed flask rotating in an oil bath. The homogeneous mass was taken up in 100 ml *n*-butanol by shaking and gentle warming. The resulting colourless sediment was collected by centrifugation, washed two times with 100 ml butanol and dissolved in water. The butanol-soluble fraction had an ultraviolet absorption characteristic for phenylpolyphosphate ester, whereas the aqueous solution of the sediment showed the ultraviolet spectrum of O^2-2'-cyclouridine (Fig. 1). The volume of the aqueous solution was increased to 300 ml and the acidity was adjusted to pH 1 with HCl. After 5 h heating at 90°, the cyclic linkage of O^2-2'-cyclouridine and the phosphodiester bonds of polyuridylic acid, if present, were hydrolyzed. After hydrolysis the ultraviolet spectrum had a maximum at 260 mμ, indicating the transformation of cyclouridine groups into the uridylic acid and spongouridylic acid groups. The solution was neutralized with 2 M $NaHCO_3$, concentrated and dialyzed against water overnight. The total amount of ultraviolet-absorbing material decreased to 50 % since mono- and oligonucleotides were removed by dialysis. After dialysis the salt concentration was so low that separation on DEAE-cellulose was possible. If the loss of oligonucleotides was to be avoided, the hydrolysis was carried out with $HClO_4$ at pH 1. After concentration, the solution was neutralized with KOH and the insoluble $KClO_4$ removed.

Chromatography on DEAE-cellulose

A column of DEAE-cellulose 25 cm × 2.5 cm was used for separating the oligonucleotides. The amount of polycondensate applied did not exceed 1500 units of absorbance. The elution gradient was produced from 1 l H_2O and 1 l 0.5 M NH_4HCO_3 of pH 8.6. The fraction of higher polymers was eluted with 1 M $(NH_4)_2CO_3$ at pH 9.6. The absorbance of the eluate was continuously recorded by an Uvicord (LKB type 4701 A), and fractions of 20 ml were collected. After the experiment, the column was regenerated with 2 M $(NH_4)_2CO_3$ at pH 9.6 and washed with water until neutral.

Separation on Sephadex G-75

For further characterization, the high polymer fractions eluted with 1 M $(NH_4)_2CO_3$ were concentrated and desalted on a column of Sephadex G-25 (150 cm × 3.2 cm) equilibrated with water. About 30 ml (100 absorbance units) were applied to the column. The polynucleotides were eluted with water and collected in fractions of 20 ml. All polymers with $n > 2$ appeared in the exclusion peak, comprising the Fractions 26–30. The salt appeared in Fraction 58. Fractions 26–30 were concentrated to 1–2 ml and applied to a column of Sephadex G-75 (145 cm × 2.8 cm) which was eluted with 0.01 M NH_4HCO_3. The fractions (15 ml) were sampled as indicated in Fig. 4 and the average molecular weight was determined in the ultracentrifuge.

The column of Sephadex G-75 was standardized with a mixture of ^3H_2O, Up, $(Up)_5$ and Blue Dextran 2000 (Pharmacia, Uppsala). ^3H_2O appeared in Fraction 63, Up in 58, $(Up)_5$ in 53 and Blue Dextran in the exclusion peak, Fraction 18. The values for all other intermediate oligonucleotides were calculated according to HOHN AND SCHALLER[9].

Determination of molecular weights

Measurements of the molecular weights were carried out according to the method of YPHANTIS[10,11] in a Beckman Model E centrifuge at 29 500 rev./min with an 8-channel middlepiece. The calculations were based on a reference measurement with a capillary cell. In each run, 4 concns. (1 %, 0.75 %, 0.5 %, 0.25 %) were measured in a solution of 0.3 M NaCl + 0.01 M Tris buffer (pH 7.4). A specific volume $V = 0.68$ was assumed. Since the results did not show systematic concentration dependence, the mean value of the 4 measurements was taken (Table I).

TABLE I

MOLECULAR WEIGHTS OF POLYSPONGOURIDYLIC ACIDS

Controls	*Calculated*	*Observed*
Up	300	360
$(Up)_5$	1550	1600
Polyspongouridylic acids		
Sephadex Fractions 44–50		2400
Sephadex Fractions 44–47		3200
Sephadex Fractions 32–44		5100

Treatment with acetic anhydride in pyridine

According to KHORANA, VIZSOLYI AND RALPH[12] pyrophosphate linkages joining oligonucleotides are cleaved by an excess of acetic anhydride in dry pyridine. Various experiments of this type were carried out, but no degradation of the polyspongouridylic acid was observed. If the dry butanol precipitate was treated with acetic anhydride in pyridine before hydrolysis the chromatogram obtained on DEAE-cellulose was the same as that without treatment.

Digestion with ribonuclease

6.6 mg polyspongouridylic acid (220 absorbance units) were incubated at 37° for 18 h with 100 μg ribonuclease (Sigma, St. Louis, 5 times crystallized). The mixture was chromatographed on Sephadex G-75. No alteration in the original chain length was observed.

Acid and alkaline hydrolysis

Polyuridylic acid was completely hydrolyzed to mononucleotides after incubation in 0.1 M HCl at 90° for 5 h. Polyspongouridylic acid treated in the same way was stable.

Samples of polyspongouridylic acid were treated with 2 M KOH after 22 h at 37°. No degradation products were chromatographically observable, whereas polyuridylic acid was hydrolyzed completely to mononucleotides.

Degradation of polyspongouridylic acid with snake-venom diesterase and bacterial alkaline monophosphatase

3 mg polyspongouridylic acid were incubated with 250 μg bacterial alkaline monophosphatase (Worthington, Freehold, N.J.) and 4 mg crude snake venom (Sigma, St. Louis) in 3 ml 0.1 M Tris buffer (pH 8.5) containing 0.001 M Mg^{2+} at 37° for 8 h. The crude snake venom contained 200 units/mg diesterase as determined according to RUZZELL AND KHORANA[13] and 1 unit/mg 5'-nucleotidase as determined according to HEPPEL AND HILMOE[14]. The digest was separated on a DEAE-cellulose column and the diagram given in Fig. 3 was obtained.

Identification of spongouridine (Su)

In butanol–water, R_F values of 0.21, 0.27 and 0.27 respectively were found for uridine, for our sample of isolated nucleoside and for a sample of Su kindly supplied by Dr. D. M. BROWN. The specific optical rotations of the same three compounds were measured with a Zeiss LEP photoelectric polarimeter, the solution concentrations having been determined spectrophotometrically. Since only small amounts were available the accuracy was low. For the isolated nucleoside $[\alpha]_D = 111°$ was found, for Su $[\alpha]_D = 131°$ and for U $[\alpha]_D = 9.6°$. The value for the isolated nucleoside is clearly consistent with the presence of Su, since the 18 % discrepancy is most likely

due to uncertainty in the concentration. Paper electrophoresis was carried out according to CODINGTON, FECHER AND FOX[15] in saturated boric acid (pH 6) at 2800 V and 45 mA. After 4 h, the nucleoside showed a homogeneous boundary at a distance of −3.2 cm from the starting point. The specimen of Su gave −3.2 cm and uridine +16 cm.

Degradation with $NaIO_4$ was studied by treating samples of the isolated nucleoside and of U with a 50-fold excess of $NaIO_4$ at 37°, and examining the ultraviolet absorption of the products separated by paper chromatography. U was completely destroyed after 1 h, yielding a tail of differently absorbing substances. Su was destroyed after 6 h.

Characterization of spongouridylic spongouridine (SupSu)

This dinucleoside phosphate SupSu was obtained in Peak II (Fig. 3) after digestion of polyspongouridylic acid with snake-venom diesterase and alkaline bacterial monophosphatase. SupSu moves slightly more slowly than uridylic uridine (UpU) on the DEAE-cellulose column. In the paper chromatography with *n*-propanol–conc. NH_3–water (55:10:35, v/v/v) SupSu migrates slightly more quickly than UpU. After hydrolysis in 1 M HCl (90°, 6 h), phenol could not be detected by Folin's reaction. Digestion with crude snake venom yielded Su. Digestion at 37° with diesterase, free of nucleotidase, in 0.01 M Tris buffer (pH 9) containing 0.001 M Mg^{2+} yielded Su and a substance which had an R_F value expected for spongouridine phosphate. By incubation of this nucleoside phosphate with 5'-nucleotidase Su was obtained.

Characterization of oligospongouridylic acids

In order to standardize the DEAE-cellulose and Sephadex columns, it was necessary to have oligouridylic acids of known chain lengths. Enzymatically prepared polyuridylic acid (1500 absorbance units) (Boehringer, Mannheim) was dissolved in 3 ml of 0.1 M NaOH and incubated at 60° for 30 min. After hydrolysis, the mixture was diluted to 10 ml and chromatographed on the same DEAE-cellulose column as mentioned before. The chain lengths were given by the sequence of the peaks and monitored by paper chromatography. As an additional control the molecular weight of $(Up)_5$ was determined in the ultracentrifuge. These fractions of oligouridylic acids were compared with fractions obtained in the DEAE-cellulose chromatography (Fig. 2) of the polycondensate.

For the determination of phosphorus in the isolated fractions the molar extinction coefficient at pH 7 for spongouridylic acid was taken as that of uridylic acid, namely 10 000. From this and the phosphate determination[16] the molar ratio P/nucleotide was calculated.

The Peaks I–III in the DEAE-cellulose chromatogram (Fig. 2) were rechromatographed on paper in the *n*-propanol–NH_3–water system. The purified fractions were compared with oligouridylic acids of known chain lengths before and after dephosphorylation with alkaline monophosphatase. The total P before digestion and the inorganic phosphate after digestion with phosphatase were determined. Peak I was mainly the starting material Up; Peak II contained spongouridylphosphate and

diphosphate besides Up and pUp. Electrophoresis in boric acid was used for the differentiation of U and Su. Peak III contained pSupSup (P/nucleoside ratio, 3:2; terminal P/total P ratio, 2:3). Peak IV was rechromatographed on DEAE-cellulose with a gradient of 0.2–0.8 M NH_4HCO_3. Not all fractions were identified. From the major peaks the following substances could be isolated by paper chromatography: (1) $(Sup)_4$ or $p(Sup)_3Su$, (2) $p(Sup)_4$, (3) $p(Sup)_5$.

Inhibition of deoxyribonuclease

8 μg of tritiated DNA (400 000 counts/min) were dissolved in 1 ml of a mixture containing 0.05 M Tris buffer (pH 7.5) and 0.01 M Mn^{2+}, 100 μg bovine serum albumin and 0.05 μg deoxyribonuclease (Worthington, Freehold, N.J.) from pancreas. The mixture was separated into two halves. One sample was mixed with 400 μg polyspongouridylate ($n = 7$–10), thus giving a polyspongouridylate/DNA ratio of 100. The samples with and without spongouridylate were incubated at 37°. At intervals of 6 min samples of 0.05 ml were withdrawn. To each sample 400 μg carrier DNA were added and precipitated with 2 ml of 0.2 M trichloroacetic acid at 0°. The precipitate was filtered and the decrease of radioactivity in the filter was determined in the liquid scintillation spectrometer (Packard Tri-Carb). The decrease of DNA per min was 500 counts/min without addition of polyspongouridylic acid and 200 counts/min in the presence of polyspongouridylic acid. In several such experiments the point of 50 % inhibition was found at a polyspongouridylic acid/DNA ratio of 100.

Inhibition of ribonuclease

Tritiated RNA was prepared from *Escherichia coli*. Another sample of ribosomal RNA, labeled with ^{14}C, was kindly given by Dr. HAUSCHILD, Tübingen. Both preparations were free of ribonuclease and stable during incubation at 37°. Samples were prepared containing 0.55 μg labeled RNA (0.018 absorbance units) in 0.8 ml of 0.05 M Tris buffer containing EDTA. One sample was used as control and immediately precipitated with trichloroacetic acid. To the others, increasing amounts of polyspongouridylic acid (0, 0.0036, 0.009 and 0.018 absorbance units) were added. To each sample $5 \cdot 10^{-4}$ μg ribonuclease stabilized with EDTA in 50 μl were added, and each sample was incubated at 37°, chilled after certain intervals and precipitated with trichloroacetic acid.

TABLE II

DEGRADATION OF TRITIATED RNA FROM *E. coli* BY RIBONUCLEASE AT 37° IN THE PRESENCE OF POLYSPONGOURIDYLIC ACID

Time of incubation (min)	*Ribonuclease in 0.8 ml (μg)*	*RNA absorbance*	*Polyspongo-uridylate absorbance*	*Counts/min in sediment*	*Difference*
0	$5 \cdot 10^{-4}$	0.018	0	5900	
5	$5 \cdot 10^{-4}$	0.018	0.018	5700	200
5	$5 \cdot 10^{-4}$	0.018	0.009	5500	400
5	$5 \cdot 10^{-4}$	0.018	0.0036	4600	1300
5	$5 \cdot 10^{-4}$	0.018	0	3100	2800

Typical results of such an experiment are given in Table II. In several such experiments with different RNA preparations and different time intervals, 50 % inhibition was found by a weight ratio polyspongouridylic acid/RNA of 1:5.

RESULTS

Disodium uridine (2')3'-phosphate and phenylpolyphosphate ester without any additional solvent were heated to 100° for 24 h, after which time a homogeneous, sometimes turbid, solution was obtained. For this reaction a phenylpolyphosphate ester preparation was used which had a molar ratio phenyl groups/phosphorus of 1.15, according to the method of preparation. At the end of the reaction phenylpolyphosphate ester can be most easily removed by addition of butanol. The polynucleotides precipitated and the phenylpolyphosphate ester remained in solution. The sediment was soluble in water and showed an ultraviolet absorption characteristic for O^2-2'-cyclouridine with a maximum at 250 mμ and a flat minimum at 235 mμ. After hydrolysis in 0.1 M HCl a mixture of oligonucleotides was obtained which showed the same absorption as polyuridylic acid with a maximum at 260 mμ (Fig. 1). The hydrolysate was then chromatographed on a column of DEAE-cellulose and the elution diagram had a number of peaks, as shown in Fig. 2.

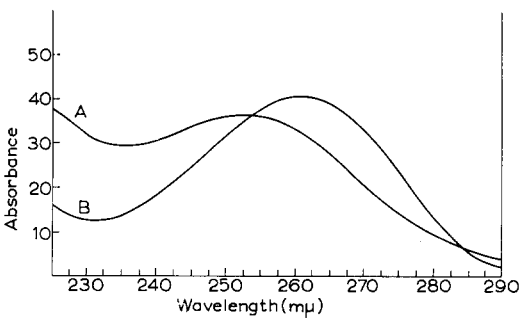


Fig. 1. Ultraviolet spectrum of polycyclouridylic acid (A) and polyuridylic acid (B).

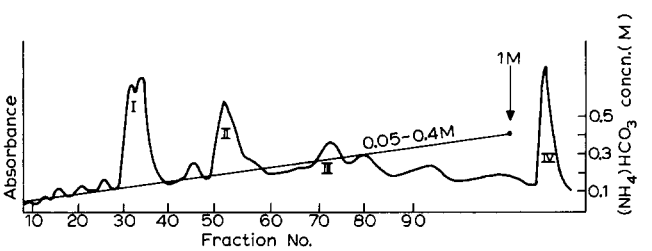


Fig. 2. Elution diagram on DEAE-cellulose of the polycondensate after hydrolysis with 0.1 M HCl and dialysis.

For the structural analysis Fraction IV was primarily used which was eluted by a gradient above 0.5 M NH_4HCO_3. This fraction contained 1.1–1.3 moles P per mole nucleoside, and could not be degraded by ribonuclease, 2 M KOH or 0.1 M HCl,

under which conditions polyuridylic acid is completely hydrolyzed. Treatment with acetic anhydride in pyridine did not reduce the molecular weight as would be expected if pyrophosphate linkages were present. However, almost complete degradation could be obtained with a mixture of crude snake-venom diesterase and alkaline bacterial monophosphatase. The enzymatic digest was chromatographed on DEAE-cellulose and 3 peaks were observed (Fig. 3). The position of Peak I on the elution

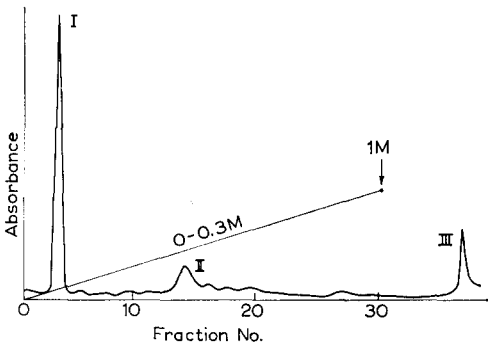
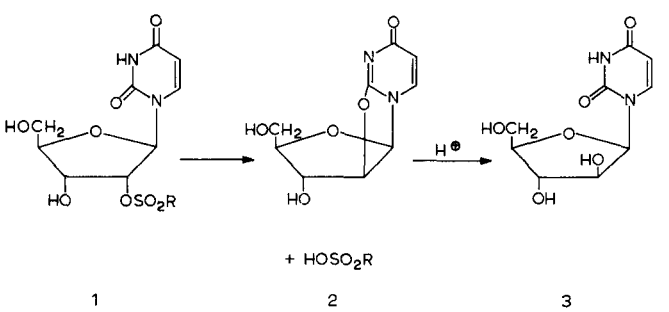


Fig. 3. Degradation products of polyspongouridylic acids after digestion with snake-venom diesterase and bacterial monophosphatase. Elution diagram on a DEAE-cellulose column.

diagram corresponded to a nucleoside, that of Peak II to a dinucleoside phosphate and that of Peak III to the starting material. The nucleoside in Peak I was identified as 3-β-D-arabinofuranosyl uracil, which has been found in sponges and which is therefore called spongouridine (Su).



Su (Formula 3) has been synthesized by BROWN, TODD AND VARADARAJAN[17] from U. According to these authors, after substitution of the 2'-hydroxyl by a sulphonyl group the O at C-2 of the uracil can attack the C-2' from the *trans* position to form an O^2-2'-cyclouridine (Formula 2). Hydrolysis in acidic solution produces arabinosyl uracil (Formula 3). Instead of the sulphonyl group a phosphate[18] or carbonate[19] group can be used for cyclization. Therefore cyclization can easily occur under the conditions of condensation by polyphosphate esters.

The identification of the isolated nucleoside as Su was effected with the following experiments. The R_F value of the nucleoside in butanol-H_2O was higher than that of U and was equal to that of a specimen of Su kindly given to us by Dr. D. M. BROWN,

London. The $[\alpha]_D$ of U is $+9.6°$, that of Su is $+131°$ and our preparation showed $[\alpha]_D = 111°$. Ribo-, arabino-, xylo- and lyxonucleosides can be distinguished by electrophoresis in boric acid at pH 6 (see ref. 13), since the stabilities of the borate complexes are different. The isolated nucleoside has the same negative mobility as Su. U reacts rapidly with periodate due to the *cis* position of the 2'- and 3'-hydroxyl groups. The isolated nucleoside reacted slowly with periodate, indicating the *trans* position of the hydroxyl groups.

The small component in Peak II of the enzymatic digest was identified as spongouridylic 5'-spongouridine by the following experiments. After hydrolysis in 1 M HCl at 90° no phenol was found which excludes the presence of a phenyl ester. By degradation with pure snake-venom phosphodiesterase, Su and spongouridine 5'-phosphate were obtained; the latter could be dephosphorylated to Su by 5'-mononucleotidase from snake venom. Since the dinucleoside phosphate is only slowly degraded by the diesterase some of it could still be found in the enzymatic digest. The undegraded polyspongouridylic acids in Peak III comprise 6–8 % of the original acid-stable material, as determined spectrophotometrically. Probably a small percentage of our preparation carries a phenyl group at the 3'-terminal phosphate since it was found to be resistent to bacterial alkaline monophosphatase as well as to the diesterase.

The diesterase of snake venom is an exonuclease requiring a free terminal 3'-hydroxyl. It specifically attacks 5'-phosphate groups of the terminal ribose or deoxyribose residues. Since more than 90 % of all phosphate bridges are split, they are located in the 5'-position. These diester bonds must be between the 5'- and 3'-positions, since the 2'-position is blocked by the O^2 of the uracil residue during the condensation. If any 2'-5'-bridges had been formed, they would not have been stable to the acid hydrolysis because of the interaction with the adjacent 3'-hydroxyl groups. Since pyrophosphate linkages should also be hydrolyzed under these conditions and degradation with acetic anhydride was not observed, the polyspongouridylic acids purified by acidic hydrolysis contain only 3'-5'-phosphate linkages like the natural polyribonucleotides. It is possible that uridylate residues not inverted in the terminal 3'-position could withstand the hydrolysis, but this is not possible in the terminal 5'-position. Probably because of this, a small amount of U was found in some preparations of polyspongouridylic acids. The P content indicates that one or both ends of the polyspongouridylates are phosphorylated. This is confirmed by the analysis of the other peaks in the DEAE-cellulose diagram (Fig. 2). All components were not identified and the characterization is not complete. By the technique described above the following spongouridylates were observed: pSup, pSupSup, $(Sup)_4$ or $p(Sup)_3Su$, $p(Sup)_4$, $p(Sup)_5$.

Since the amounts of higher spongouridylates are small and their separation is difficult, the average chain length of this material was determined on a standardized column of Sephadex G-75. For standardization $K_D = 0$ was determined with Blue Dextran and $K_D = 1$ with ^3H_2O. As further reference points the K_D values for Up and $(Up)_5$ were determined. According to HOHN AND SCHALLER[9], the K_D values for any chain length can be calculated and the values found in the present study are given in Fig. 4. The maximum of the distribution curve is near $n = 7$, but spongouridylates with $n = 10$ to 20 were also present. The average chain lengths found by the comparison of the elution velocities were monitored by molecular weight de-

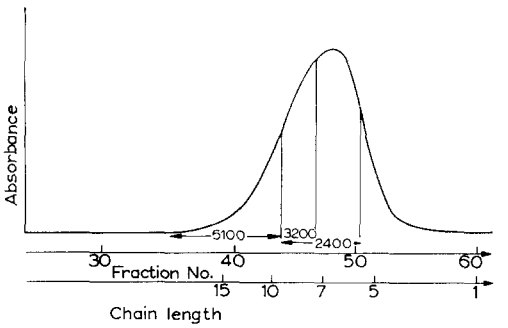


Fig. 4. Elution diagram of polyspongouridylic acids on Sephadex G-75. Chain lengths calculated according to HOHN AND SCHALLER[9]. Molecular weights determined by ultracentrifugation.

terminations in the ultracentrifuge according to the equilibrium method of YPHANTIS[10,11]. For the medium fraction with $n = 6$–9, an average molecular weight of 2400 was found corresponding to $n = 8$; for the top fraction comprising $n = 10$–20 a molecular weight of 5100 was found corresponding to $n = 17$ (Table I). To determine the yields, fractions of known chain length were measured in the spectrophotometer and the values given in Table III were obtained. Generally in this type of

TABLE III

YIELDS OF POLYSPONGOURIDYLIC ACID FROM 1 g URIDYLIC ACID (24 000 ABSORBANCE UNITS)

n	*Absorbance units*	*mg*
4	160	5.3
5	128	4.3
6	92	3.1
>8	43	1.4

condensation, the amounts of polymer products follow a Poisson distribution. Using a large amount of starting material polyspongonucleotides of high molecular weight should be expected in μg amounts sufficient for biological experiments.

The α side of arabinonucleosides is very similar to the α side of 2'-deoxyribonucleosides because of the *trans* position of the 2'-hydroxyl (Formula 1). Enzymes are known which attack arabinonucleosides instead of 2-deoxyribonucleosides, but not ribonucleosides[20]. Polyarabinonucleotides occupy a position intermediate between RNA and DNA. The resistance of their phosphate linkages to alkali and ribonuclease resembles that of DNA; however, in acidic solution the stability of the *N*-glycosidic linkage in the polyarabinonucleotides resembles that of RNA rather than that of DNA because of the inductive influence of the 2'-hydroxyl.

We studied the behaviour of the polyarabinonucleotides towards enzymes specific for DNA and RNA. It was found that polyspongouridylic acids were not degraded by pancreatic deoxyribonuclease, but some competitive inhibition of this endonuclease was observed. In the presence of a 100-fold excess of polyspongouridylate ($n = 7$–10) the rate of degradation of DNA was decreased by 50 %. Poly-

spongouridylic acids have a low susceptibility to deoxyribonuclease. A high susceptibility was, however, found to pancreatic ribonuclease. The hydrolysis of RNA by the enzyme was followed by measuring the increase of acid-soluble oligonucleotides after precipitation of the labeled RNA with $HClO_4$. At the ratio polyspongouridylic acid/RNA 1:1, the inhibition was over 90 %; at the ratio 1:5 the inhibition was still 50 %. Therefore it may be concluded that oligospongouridylates ($n = 7$–10) have a susceptibility to pancreatic ribonuclease which is 5 times higher than that of the high molecular weight RNA. The inhibition depends on the chain length. Smaller oligonucleotides and monomeric spongouridylic acid inhibit much more weakly. The inhibition constants with polyspongouridylates of different chain lengths have not yet been determined quantitatively.

The biochemical properties of polyspongouridylates were further investigated in preliminary experiments on the adaptation of phenylalanine-tRNA on ribosomes, using it instead of polyuridylic acid. These experiments were performed by H. BERGER in the laboratory of Dr. H. MATTHAEI in Göttingen. Polyspongouridylates fractionated on Sephadex G-75 were dephosphorylated by alkaline bacterial phosphatase and purified by paper chromatography in 1 M ammonium acetate–96 % ethanol (1:1;v/v), pH 7.2. Fractions with $R_F = 0.25$–0.63 were used for the experiment. Polyuridylic acid (Miles Chemical Co., Clifton, N.J.) and oligouridylates obtained from it by alkaline degradation were used for comparison. The latter were also de-

TABLE IV

ADAPTATION OF PHENYLALANINE-tRNA ON RIBOSOMES IN THE PRESENCE OF POLYURIDYLIC ACID AND POLYSPONGOURIDYLIC ACID

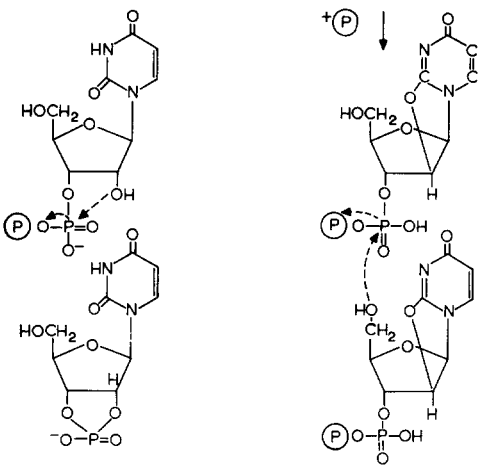
Expt. No.	*mRNA added* (μg)		*Ribosomes added* ($\mu\mu moles$)	*Adapted Phe-tRNA** (*counts/min*)
I	Control	—	—	33
	Control	—	50	537
	Polyspongouridylic acid n about 7	0.33	50	1026
	Polyspongouridylic acid n about 7	0.66	50	1062
	Polyspongouridylic acid n about 7	1.65	50	918
	Polyuridylic acid n about 10	0.36	50	4243
	Polyuridylic acid n about 10	0.72	50	4193
	Polyuridylic acid n about 10	1.80	50	4122
	Polyuridylic acid n about 100	10	50	7291
II	Control	—	—	49
	Control	—	50	376
	Polyspongouridylic acid n about 7	6.6	50	788
	Polyuridylic acid $n < 10$	7.8	50	619
				567

* 20 μg phenylalanine-tRNA for each experiment in a total volume of 0.1 ml. Procedure according to MATTHAEI *et al.*[22,23].

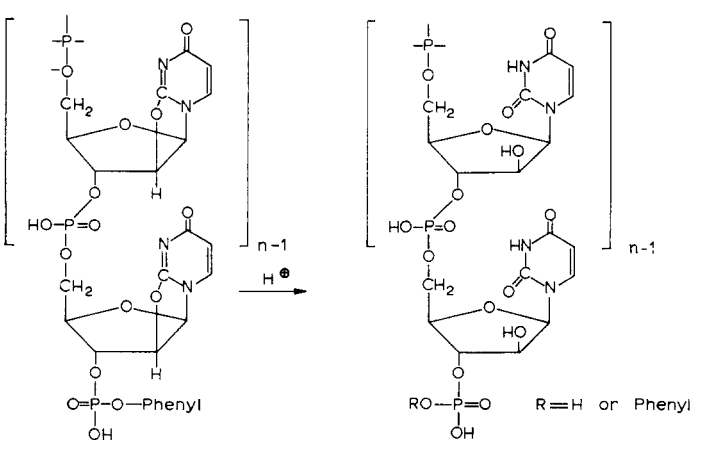
phosphorylated and purified by chromatography. The oligouridylic fraction with $n < 10$ had an R_F value of 0; the preparation with n approx. 10, $R_F = 0.25$–0.64. A stimulation of the adaptation was observed which was about twice that of the blank value (Table IV). From these preliminary experiments it cannot be concluded whether polyspongouridylic acid has the same or a weaker stimulating effect than polyuridylic acid of the corresponding chain length.

DISCUSSION

The formation of polyspongouridylic acid from uridylic acid occurs in several steps. Uridine 2'(3')-phosphate reacts with phenylpolyphosphate ester to form uridine polyphosphate ester. This enables the cyclization to 2',3'-cyclophosphate (Formula 4)



4 5



6 7

which was isolated in short-term experiments under mild conditions. Substitution of the 2'-hydroxyl permits a nucleophilic attack on C-2' by the oxygen of uracil to form O^2-2'-cyclouridine 3'-phosphate. By further phosphorylation of the 3'-phosphate a nucleophilic attack at the 5'-hydroxyl of the next nucleotide is possible (Formula 5). A chain of cyclouridylates (Formula 6) is formed which can be converted to polyspongouridylic acids (Formula 7) by acid hydrolysis.

A similar reaction can be effected by ethylpolyphosphate esters, but it seems that the inversion is more complete with phenylpolyphosphate ester. The use of phenylpolyphosphate ester has further advantages, in that its preparation is simple and easily reproducible, its reactions lead only to phosphorylation, and phenylation by breakage of the C–O bond has not been observed. Further, its solubility in water is low, allowing convenient removal by extraction with organic solvents.

The structural resemblance between the polyarabinonucleotides and polydeoxyribonucleotides does not seem to be very high, as indicated by the low affinity to deoxyribonuclease. A greater resemblance to RNA is indicated by the high susceptibility of polyspongouridylic acid to ribonuclease and its ability to function as mRNA for ribosomes. This latter effect has to be studied in more detail. Such studies may lead to more information about the function of the sugar moiety during the adaptation process.

The high stability of the polyarabinonucleotides in acid and alkali is interesting. In an earlier publication[21] a hypothesis was presented concerning the possible origin of nucleic acids in prebiological systems. The stability of the polyarabinonucleotides and their smooth formation from mononucleotides and polyphosphates suggests that polynucleotides of this type might have been the first matrices which induced the formation of further polynucleotides from monomers or oligomers. The intermediate position of the polyarabinonucleotides between RNA and DNA suggests further that they might have been a starting point for the evolution of both types of nucleic acids.

ACKNOWLEDGEMENT

We thank Miss CH. ULRICH for carrying out some of the control experiments.

REFERENCES

1 G. SCHRAMM, H. GRÖTSCH AND W. POLLMANN, *Angew. Chem.*, 74 (1962) 53.
2 K. LANGHELD, *Chem. Ber.*, 43 (1910) 1857.
3 K. LANGHELD, *Chem. Ber.*, 44 (1911) 2076.
4 N. K. KOCHETKOV, E. I. BUDOWSKY, V. D. DOMKIN AND A. N. KHROMOV–BORISSOW, *Biochim. Biophys. Acta*, 80 (1964) 145.
5 E. P. GOTTICH AND O. I. SLUTSKY, *Biochim. Biophys. Acta*, 87 (1964) 163.
6 F. N. HAYES AND E. HANSBURY, *J. Am. Chem. Soc.*, 86 (1964) 4172.
7 D. BEYERSMANN, *Z. Naturforsch.*, in the press.
8 G. SCHRAMM AND H. BERGER, *Z. Naturforsch.*, in the press.
9 TH. HOHN AND H. SCHALLER, *Biochim. Biophys. Acta*, 138 (1967) 466.
10 D. YPHANTIS, *Biochemistry*, 3 (1964) 297.
11 D. YPHANTIS, *Ann. N.Y. Acad. Sci.*, 88 (1960) 586.
12 H. G. KHORANA, J. P. VIZSOLYI AND R. K. RALPH, *J. Am. Chem. Soc.*, 84 (1962) 414.
13 W. E. RUZZELL AND H. G. KHORANA, *J. Biol. Chem.*, 234 (1959) 2105.
14 I. A. HEPPEL AND R. J. HILMOE, *J. Biol. Chem.*, 188 (1951) 665.

15 J. F. CODINGTON, R. FECHER AND J. J. FOX, *J. Am. Chem. Soc.*, 82 (1961) 2794.
16 J. B. MARTIN AND D. M. DOTY, *Anal. Chem.*, 21 (1949) 965.
17 D. M. BROWN, A. R. TODD AND S. VARADARAJAN, *J. Chem. Soc.*, (1956) 2388.
18 E. R. WALWICK, W. K. ROBERTS AND C. A. DEKKER, *Proc. Chem. Soc.*, (1959) 84.
19 A. HAMPTON AND A. W. NICHOL, *Biochemistry*, 5 (1966) 2076.
20 W. W. LEE, A. BENITEZ, L. GOODMAN AND B. R. BAKER, *J. Am. Chem. Soc.*, 82 (1960) 2648.
21 G. SCHRAMM, *Synthesis of nucleosides and polynucleotides with metaphosphate esters in origins of prebiological systems and of their matrices*, Academic Press, New York, 1965, p. 299.
22 J. H. MATTHAEI, H. P. VOIGT, G. HELLER, R. NETH, G. SCHICK, H. KRIBLER, F. AMELUNXEN, G. SANDER AND A. PARMEGGANI, *Cold Spring Harbor Symp. Quant. Biol.*, 31 (1966) 25.
23 J. H. MATTHAEI, G. HELLER, H. P. VOIGT, R. NETH, G. SCHICK AND H. KRIBLER, *Proc. 3rd Meeting Europ. Biochem. Soc. Warsaw, 1966*, Academic Press, New York - Polish Scientific Publishers, Warsaw, 1967, p. 233.