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Synthesis of 2′-5′ Linked Oligouridylates in Aqueous Medium Using the Pd^{2+} Ion

Hiroaki Sawai* and Masaji Ohno

Faculty of Pharmaceutical Sciences, University of Tokyo
Hongo, Bunkyo-ku, Tokyo 113, Japan

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Oligouridylates up to the pentamer were obtained by the polymerization of uridine-5′-phosphorimidazolide in aqueous solution using Pb^{2+} ion catalyst. The resulting oligouridylates were fully characterized by sequential enzyme and alkaline digestions. The internucleotide linkages of the oligomers were mainly 2′-5′. The yields of the 2′-5′ linked dimer, trimer, tetramer and pentamer were 14.2, 6.0, 2.0 and 0.4%, respectively.

Keywords——oligouridylates; 2′-5′ internucleotide linkage; urdine-2′-phosphorimidazolide; polymerization; Pb^{2+} ion; enzymatic degradation; QAE-Sephadex column chromatography; paper chromatography

Recently unusual 2′-5′ linked 5′-triphosphoryl oligoadenylates (2-5 A) have been isolated from interferon-treated cells; these were the first 2′-5′ linked oligonucleotides to be isolated from natural sources.[1] 2-5 A has strong inhibitory activity on protein biosynthesis.[1,2] 2-5 A synthetase, which synthesizes 2-5 A from ATP, aslo promotes the formation of 2′-5′ linked co-oligonucleotides with adenosine triphosphate and other nucleotides.[3] The biological action of the co-oligonucleotides and other 2′-5′ linked homooligonucleotides is unknown. Therefore, we have undertaken the chemical synthesis of various structurally modified 2′-5′ linked oligonucleotides in order to study the effects of different groups in the base moiety and of different internucleotide linkages. In this paper, we wish to report the preparation of oligouridylates with 2′-5′ linkages.

Various groups have synthesized 2-5 A and its core $A^{2'}p^{5'}A^{2'}p^{5'}A$ chemically, mainly by the triester method.[4] We have reported that 2′-5′ linked oligoadenylates with a 5′-phosphate end can be prepared from adenosine-5′-phosphorimidazolide (ImpA) in an aqueous medium by using divalent metal ions such as Co^{2+}, Zn^{2+} and Pb^{2+}.[5]

We have shown that the procedure using the Pb^{2+} ion can be used as a large-scale preparative method for various 2′-5′ linked oligoadenylates up to the pentamer.[6] Substantial amounts of 2′-5′ linked diadenylate and triadenylate with 5′-phosphate ends were obtained along with related structural isomers in a one-pot reaction. The 2′-5′ linked triadenylate thus obtained was easily converted to the biologically active 2-5 A.[7] A preliminary study indicated that a similar method is applicable for the synthesis of 2′-5′ linked oligouridylates from imidazolide (ImpU).[8] However no detailed characterization of the polymerization of ImpU has been carried out from a synthetic point. We prepared various oligouridylates with 2′-5′ internucleotide linkages from ImpU by using the Pb^{2+} ion and fully characterized their structures by sequential enzymatic tests.

Results and Discussion

ImpU was prepared from uridine-5′-monophosphate (pU) and imidazole using a condensing agent. The phosphorimidazolide linkage of ImpU is very susceptible to nucleophilic attack. Thus, ImpU is hydrolyzed easily to pU in aqueous solution. Pb^{2+} promotes the polymerization of ImpU in aqueous medium. Fine white precipitates were formed by the addition of lead nitrate to a solution of ImpU. ImpU coordinates to the Pb^{2+} ion to give the Pb^{2+}–ImpU

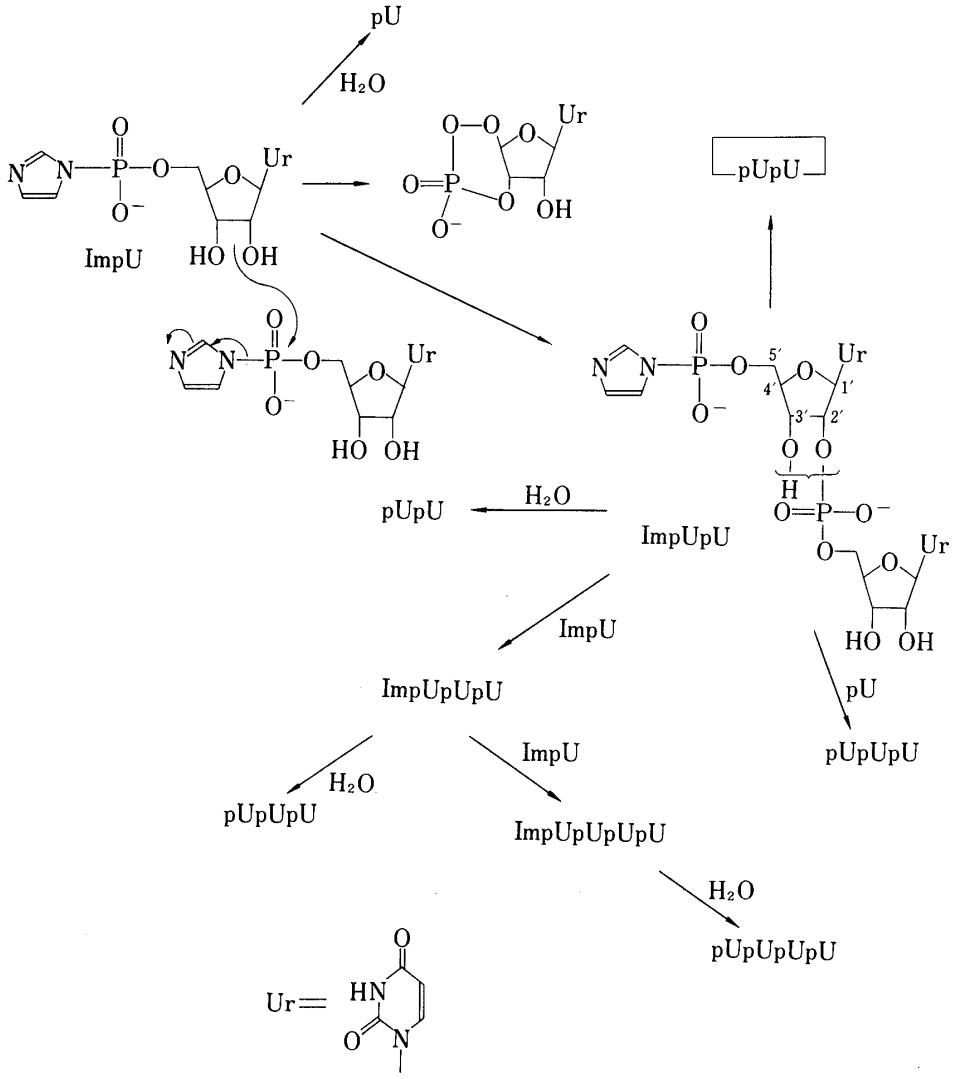

Chart 1. Interncleotide Linkage Formation and Hydrolysis of Phosphorimidazolide

complex which precipitates partly because of its low solubility. The polymerization reaction was conducted in suspension with stirring at room temperature. As the reaction progressed, the formation of oligouridylates took place and the starting ImpU disappeared in 5 days. The condensation reaction proceeded together with a simple hydrolysis reaction (Chart 1). Pyrophosphate formation took place as a minor reaction (Chart 2).

The reaction mixture became homogeneous on treatment with Versen-01® buffer. Versen-01® coordinates to Pb^{2+} forming Pb^{2+}–Versen-01® complex, which was confirmed to be present by high pressure liquid chromatography (HPLC). The products were separated by QAE-Sephadex A-25 anion exchange column chromatography. Stepwise linear gradient elution of the column gave a good separation of linkage isomers of oligouridylates. The elution pattern is shown in Fig. 1. The products were further purified by paper chromatography when necessary. The distribution and yield of the products are listed in Table I along with the assigned structure and the hyperchromicity.

Oligouridylates up to the pentamer were obtained in this reaction. A 2′-5′ internucleotide linkage was preferentially formed. The yields of fully 2′-5′ linked diuridylate, triuridylate, tetrauridylate and pentauridylate were 14.2, 6.0, 2.0 and 0.4%, respectively. The dimer and the trimer in peak 7 and peak 12, respectively, were practically pure. They were insensitive to nuclease P_1 (N. P_1) and pancreatic ribonuclease, which degrade 3′-5′ internucleotide linkages.

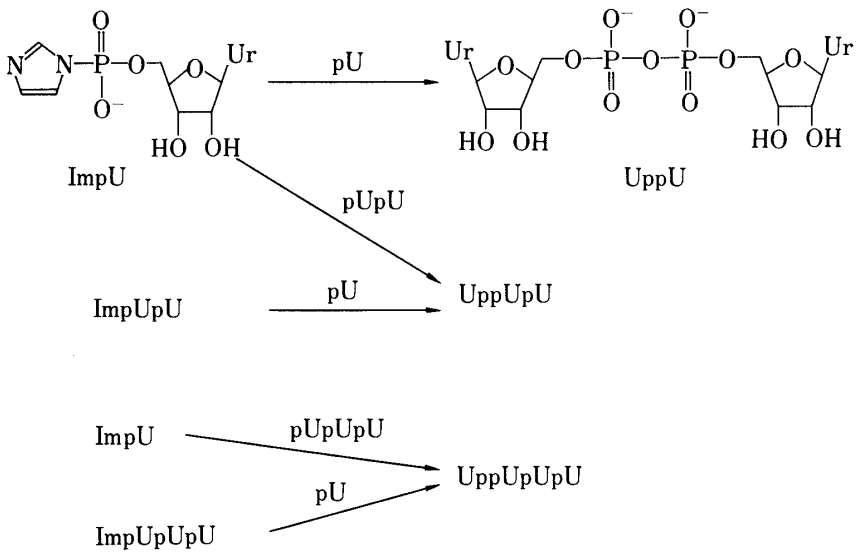

Chart 2. Pyrophosphate Formation (Minor Reaction)

Incubation of the oligouridylates with venom phosphodiesterase (VPDase) or alkaline solution degraded their internucleotide linkages. The chain length and the 2′-5′ internucleotide linkages were characterized by degradation of the oligouridylates with several enzymes and alkaline solution.

The 3′-5′ linked dimer and trimer were present in peak 8 and peak 14, respectively. The yields were about one-tenth of those of the corresponding 2′-5′ linked isomers. They were characterized by cochromatography with authentic samples and N. P_1 digestion.

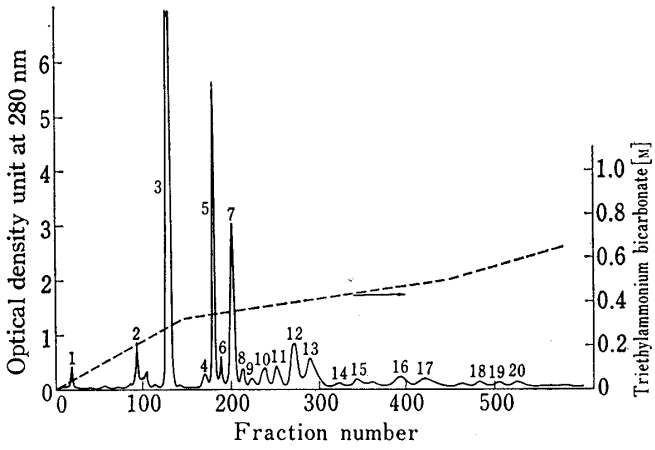

Fig. 1. Elution Curve of Oligouridylates Obtained from ImpU by Using Pb^{2+}

Two linkage isomers of triuridylates, $pU^{2'}p^{5'}U^{3'}p^{5'}U$ and $pU^{3'}p^{5'}U^{2'}p^{5'}U$, were present in peak 13. The compound $pU^{2'}p^{5'}U^{3'}p^{5'}U$ gave, after incubation with bacterial alkaline phosphatase (BAP), $U^{2'}p^{5'}U^{3'}p^{5'}U$ which was degraded by N. P_1 to $U^{2'}p^{5'}U$ and pU. On the other hand, digestion of $pU^{3'}p^{5'}U^{2'}p^{5'}U$ with BAP gave $U^{3'}p^{5'}U^{2'}p^{5'}U$, which was degraded by N. P_1 to U and $pU^{2'}p^{5'}U$. The yield of $pU^{2'}p^{5'}U^{3'}p^{5'}U$ was several times higher than that of $pU^{3'}p^{5'}U^{2'}p^{5'}U$.

The linkage isomer of tetrauridylates with one 3′-5′ linkage and two 2′-5′ linkages were formed in small amounts (peak 17). Very small amounts of other linkage isomers of the tetramer and pentamer were present in peaks 18, 19 and 20, but their purification and characterization were not attempted.

Cyclic dinucleotides were obtained in more than 10% yield (peaks 5 and 6). Among the possible three linkage isomers, 3′-5′ linked cyclic dimer was present in a large amount. In contrast to the linear oligouridylates series, 3′-5′ linkage is predominant in the cyclic series. Examination of molecular models suggested that cyclic dinucleotides would favor the 3′-5′ linkage rather than the 2′-5′ linkage. The cyclic diuridylate with two 3′-5′ linkages was characterized by its resistance to BAP and by degradation with N. P_1 to pU. The 3′-5′, 2′-5′ cyclic diuridylates were insensitive to BAP but were hydrolyzed with N. P_1 to $pU^{2'}p^{5'}U$.

TABLE I. Oligouridylates obtained from ImpU

Peak No	ODU_{262}	h[a]	Assigned structure	Yield (%)[b]
1	40		U	0.8
2	142		3′,5′ cyclic UMP	2.8
3	1637		pU	32.8
4	42(52)[d]		UppU	0.8
5	565	1.06	—pUpU— (3′–5′, 3′–5′)	8.6
			—pUpU— (3′–5′, 2′–5′)	3.2
6	80	1.04	—pUpU— (2′–5′, 2′–5′)	1.6
7	660	1.08	$pU^{2'}p^{5'}U$	14.2
8	72	1.07	$pU^{3'}p^{5'}U$	1.5
9	30(40)[d]	1.14	$UppU^{2'}p^{5'}U$	0.6
10	136		unidentified	2.7[c]
11	125		unidentified	2.5[c]
12	271	1.10	$pU^{2'}p^{5'}U^{2'}p^{5'}U$	6.0
13	274	1.07	$pU^{2'}p^{5'}U^{3'}p^{5'}U$	5.0
			$pU^{3'}p^{5'}U^{2'}p^{5'}U$	0.7
14	16(36)[d]	1.09	$pU^{3'}p^{5'}U^{3'}p^{5'}U$	0.3
15	18(61)[d]		$UppU^{2'}p^{5'}U^{2'}p^{5'}U$	0.3
16	100(116)[d]	1.15	$pU^{2'}p^{5'}U^{2'}p^{5'}U^{2'}p^{5'}U$	2.0
17	111		$pU^{2'}p^{5'}U^{2'}p^{5'}U^{3'}p^{5'}U$	0.3[c]
			$pU^{2'}p^{5'}U^{3'}p^{5'}U^{2'}p^{5'}U$	1.7[c]
			$pU^{3'}p^{5'}U^{2'}p^{5'}U^{2'}p^{5'}U$	0.2[c]
18	43		unidentified	0.8[c]
19	22(37)[d]		$pU^{2'}p^{5'}U^{2'}p^{5'}U^{2'}p^{5'}U^{2'}p^{5'}U$	0.4[c]
20	32		$(pU)_5$	0.5[c]

a) Hyperchromicity was obtained from the ratio of the UV absorption at 262 nm after and before alkaline hydrolysis of each compound.
b) Yield was determined from the UV_{262} absorption after allowing for the hyperchromicity of each oligouridylate. Total ODU_{262} of the starting ImpU was 5015 (0.5 mmol).
c) Hyperchromicity correction was not made in these cases.
d) Including the ODU_{262} of by-products in addition to the identified compound.

The 2′-5′, 2′-5′ linked isomer was insensitive to BAP and N. P_1 but was degraded by alkaline solution and VPDase.

The compounds with a pyrophosphate bond, UppU, $UppU^{2'}p^{5'}U$ and $UppU^{2'}p^{5'}U^{2'}p^{5'}U$, were obtained as minor by-products. They were insensitive to BAP and N. P_1. The hydrolysis of $UppU^{2'}p^{5'}U$ in 0.5 M NaOH solution gave UppUp and U. UppUp was characterized by digestion with BAP to UppU. In a similar manner, $UppU^{2'}p^{5'}U^{2'}p^{5'}U$ was degraded in 0.5 M NaOH solution to UppUp, Up and U. They could be prepared by pyrophosphate formation from ImpU with $pU^{2'}p^{5'}U$ or $pU^{2'}p^{5'}U^{2'}p^{5'}U$.

Uridine, 3′-5′ cyclic uridine monophosphate, pU and UppU were characterized on the basis of their elution positions from the column and by comparison of their paper chromatography and HPLC properties with those of authentic samples. Intramolecular phosphodiester bond formation of ImpU gave 3′-5′ cyclic UMP.

The results obtained so far do not establish the mechanistic role of the Pd^{2+} ion in the polymerization of ImpU. However, we believe that the polymerization of ImpU proceeds in the coordination sphere of the Pb^{2+} ion. An NMR study of ImpA in the presence of a small amount of Pb^{2+} showed that ImpA coordinates to the Pb^{2+} ion at the phosphate and base

moieties.[8] In the case of ImpU, similar complex formation is likely to occur, though the coordination of the base moiety is different. The Pb^{2+} ion would organize ImpU by coordination and enhance the nucleophilicity of the 2′-OH group of ImpU so as to facilitate internucleotide linkage formation.

The polymerization reaction was conducted in suspension. However, the formation of the insoluble Pb^{2+}–ImpU complex is not necessary for the polymerization. The Hg^{2+} ion forms an insoluble Hg^{2+}–ImpU complex, but does not promote the polymerization at all. On the other hand, the Co^{2+} ion promotes the polymerization of ImpU in a homogeneous aqueous solution.[8]

The present reaction results in preferential formation of 2′-5′ linked linear oligouridylates. The yield and the selectivity of 2′-5′ internucleotide linkage are lower than those of the oligoadenylates described previously.[6] The yields of 2′-5′ linked oligouridylates obtained so far are not satisfactory, and further work on this method is necessary. However, this procedure is very simple, and the internucleotide linkage formation takes place selectively without using any protecting group. Thus, this method provides a simple synthetic route to short-chain oligouridylates with 2′-5′ internucleotide linkages.

Experimental

Materials——Uridine-5′-monophosphate sodium salt was from Yamasa. Imidazole was purchased from Tokyo Kasei and recrystallized from benzene. Triphenylphosphine, Versen-01® trisodium salt and lead nitrate were obtained commercially. 2,2′-Dipyridyldisulfide was prepared by a modification of the published procedure.[10] Bacterial alkaline phosphatase (BAPF) and venom phosphodiesterase (VPDase) were from Worthington, and nuclease P_1 (N. P_1) was from Yamasa.

Preparation of ImpU——ImpU was prepared by a modification of the method of Lohrmann and Orgel.[11] Imidazolium salt of pU was prepared by passing the pU sodium salt through a Dowex 50W-X8 (imidazolium form) column and the eluate was lyophilized. Triphenylphosphine (790 mg, 3 mmol) and 2,2′-dipyridyldisulfide (660 mg, 3 mmol) were added to a solution of pU-imidazolium (450 mg, 1 mmol), imidazole (680 mg, 10 mmol), triethylamine (1 ml) and tri-*n*-octylamine (0.5 ml) in dry dimethylformamide (10 ml) with stirring. The reaction mixture was kept at room temperature for 2 h with stirring. After the completion of the ImpU formation (as checked by Silica gel TLC using a 2-propanol–concentrated ammonia–water (7 : 2 : 1) solvent system), the reaction mixture was poured into a solution of acetone (200 ml), ether (100 ml), triethylamine (10 ml) and acetone saturated with sodium perchlorate (1 ml) with stirring to precipitate ImpU as its sodium salt. The white precipitates were collected with a glass filter, washed with dry acetone and ether several times under a slow stream of dry nitrogen and dried over P_2O_5 under vacuum. ImpU thus obtained was practically pure and the yield was more than 90% (440 mg, 9020 ODU_{262}). Analysis of the freshly prepared ImpU by HPLC showed that ImpU was the only UV-absorbing compound. ImpU can be stored in a refrigerated desiccator.

Paper Chromatography and High Pressure Liquid Chromatography (HPLC)——Paper chromatography was performed on Whatman 3MM paper by the descending technique. The solvent systems (1) 1-propanol–concentrated ammonia–water (55 : 10 : 35) and (2) saturated ammonium acetate–0.1 M sadium acetate (pH 6.5)–2-propanol (79 : 19 : 2) were used as eluents. The chromatographic mobilities of the compounds are listed in Table II.

HPLC was carried out with a Hitachi 635 apparatus using a RPC-5 column (4 mmϕ × 25 cm). RPC-5 was originally supplied by Miles laboratory, but is no longer in production. We prepared RPC-5 from Adogen 464 and granular polychlorotrifluoroethylene according to the procedure described by Pearson and Kelmers.[12] Elution was carried out with a linear gradient of NaCl solution (0.001 M—0.5 M) buffered with 0.0025 M Tris–HCl at pH 7.5. The compounds were monitored in terms of UV absorption at 260 nm. Linkage isomers of oligouridylates with 2′-5′ and 3′-5′ internucleotide bonds were separated very well by HPLC with the RPC-5 column. The results of analysis of linkage isomers of oligonucleotides and mononucleotides by HPLC with an RPC-5 column will be published elsewhere.

Polymerization of ImpU——To the aqueous solution (9.5 ml) of ImpU (245 mg, 5015 ODU_{262}, ~0.5 mmol) in imidazole buffer (0.2 M, pH 6.5), 0.5 ml of 0.25 M lead nitrate solution was added with stirring. White precipitates were formed. The reaction mixture was kept for 5 days at 20°C with stirring. Addition of 0.6 ml of 0.25 M Versen-01® buffer to the reaction mixture caused the solution to become homogeneous. The formation of Pb^{2+}-Versen-01® complex took place completely. HPLC of a small portion of the solution (2 μl) showed the formation of oligouridylates and Pb^{2+}-Versen-01® complex. The starting ImpU disappeared

in 5 days. The mixture was diluted with water (50 ml) and applied to a QAE-Sephadex A-25 anion exchange column (25 mmϕ × 45 cm) in the bicarbonate form. The column was eluted with a stepwise linear gradient of triethylammonium bicarbonate buffer, [(1) H_2O (1 l)—1/3 M (1 l); (2) 1/3 M (2 l)—1/2 M (2 l); (3) 1/2 M (1 l)—2/3 M (1 l)]. The eluate was fractionated (one 14 ml fraction every ten min). UV absorption of each fraction was measured at 280 nm. The UV absorbing fractions were pooled and evaporated to dryness under reduced pressure below 30°C. The carbonate-free residue was dissolved in water to give the oligouridylate as its triethylammonium salt. The products were further purified by paper chromatography using solvent system 1 when necessary.

Table II. Chromatographic Mobilities of the Compounds

Compound	*Rf* value relative to pU	
	Solv. 1	Solv. 2
U	1.50	0.92
3′-5′ cyclic UMP	1.43	0.75
ImpU	1.53	
pU	1.00	1.00
UppU	0.98	0.88
$\boxed{\text{—pUpU—}}$ (3′–5′, 3′–5′)	1.25	0.62
$\boxed{\text{—pUpU—}}$ (2′–5′, 2′–5′)	1.20	0.73
$pU^{2'}p^{5'}U$	0.86	0.85
$pU^{3'}p^{5'}U$	0.86	0.84
pUp	0.78	1.05
$UppU^{2'}p^{5'}U$	0.77	0.82
$pU^{2'}p^{5'}U^{2'}p^{5'}U$	0.75	0.69
$pU^{2'}p^{5'}U^{3'}p^{5'}U$	0.73	0.68
$pU^{3'}p^{5'}U^{2'}p^{5'}U$	0.73	0.67
$pU^{3'}p^{5'}U^{3'}p^{5'}U$	0.72	0.70
$pU^{2'}p^{5'}Up$	0.57	0.96
$pU^{2'}p^{5'}U^{2'}p^{5'}U^{2'}p^{5'}U$	0.48	0.63
$pU^{2'}p^{5'}U^{3'}p^{5'}U^{2'}p^{5'}U$	0.50	0.62
$pU^{2'}p^{5'}U^{2'}p^{5'}U^{2'}p^{5'}U^{2'}p^{5'}U$	0.34	0.50

Characterization of the Products——Identification of the products was carried out by means of sequential enzyme digestions. About 30 μl of each product (10—20 ODU_{262}) was put on the 3 MM paper and chromatographed on solvent system 1. The UV-absorbing part was cut out and material was eluted from the paper with water. The eluate was concentrated and incubated with BAP to remove the 5′-phosphate end. The digested solution was subjected to paper chromatography with solvent system 1. The UV absorbing part was eluted from the paper, digested with N. P_1 to degrade 3′-5′ internucleotide linkages and subjected to paper chromatography. The UV absorbing part eluted from the paper was incubated with VPDase and 0.5 M NaOH solution to degrade 2′-5′ internucleotide linkages. The degraded products were analyzed by HPLC.

Digestion with BAP was carried out for 2.5 h at 37°C in a mixture (50 μl) containing the substrate (2—15 ODU_{262}), 0.1 M Tris-HCl (pH 8.05), 0.001 M $MgCl_2$ and 0.1 unit of the enzyme.

Digestion with N. P_1 was performed for 2.5 h at 37°C in a mixture (50 μl) containing the substrate (2—10 ODU_{262}), 0.006 M Veronal-acetate buffer (pH 5.75) and enzyme solution (5 μg in 5 μl).

Incubation with VPDase was carried out for 2.5 h at 37°C in a mixture (50 μl) containing the substrate (1—5 ODU_{262}), 0.01 M Tris-acetate (pH 8.8), 0.01 M $MgCl_2$ and enzyme solution (0.1 unit).

Alkaline hydrolysis was performed for 1 day at room temperature in a mixture (50 μl) containing the substrate (1—5 ODU) in 0.5 M NaOH solution.

We checked that the reaction time and the quantity of enzymes were sufficient for complete digestion using $pU^{3'}p^{5'}U$ and $pU^{2'}p^{5'}U$ as model substrates.

2′-5′ Linked oligouridylates from dimer to pentamer were characterized by sequential enzyme and alkaline hydrolysis as follows.

$$pU^{2'}(p^{5'}U^{2'})_np^{5'}U \xrightarrow{\text{N. P}_1} \times$$

$$pU^{2'}(p^{5'}U^{2'})_np^{5'}U \xrightarrow{\text{NaOH}} pUp + Up + U\,(1 : n : 1)$$

$$pU^{2'}(p^{5'}U^{2'})_np^{5'}U \xrightarrow{\text{BAP}} U^{2'}(p^{5'}U^{2'})_np^{5}U \xrightarrow{\text{N. P}_1}$$

$$U^{2'}(p^{5'}U^{2'})_np^{5}U \xrightarrow{\text{VPDase}} U + pU\,(1 : n+1)$$

$$U^{2'}(p^{5'}U^{2'})_np^{5}U \xrightarrow{\text{NaOH}} Up + U\,(n+1 : 1)$$

$n = 0, 1, 2, 3$

Oligouridylates with 3′-5′ linkages were identified by comparison of their paper chromatograms with those of authentic samples which were prepared by partial hydrolysis of poly U with pork liver nuclease.[13] The structure was further confirmed by enzymatic degradation.

$$pU^{3'}(p^{5'}U^{3'})_np^{5'}U \xrightarrow{\text{N. P}_1} pU$$

$$pU^{3'}(p^{5'}U^{3'})_np^{5'}U \xrightarrow{\text{NaOH}} pUp + Up + U(1 : n : 1)$$

$$pU^{3'}(p^{5'}U^{3'})_np^{5'}U \xrightarrow{\text{BAP}} U^{3'}(p^{5'}U^{3'})_np^{5'}U \xrightarrow{\text{N. P}_1} U + pU(1 : n+1)$$

$n = 0, 1$

Two linkage isomers of triuridylates which contain one 3′-5′ linkage and one 2′-5′ linkage were identified as follows.

$$pU^{2'}p^{5'}U^{3'}p^{5'}U \xrightarrow{\text{N. P}_1} pU^{2'}P^{5'}U + pU\,(1 : 1)$$

$$pU^{2'}p^{5'}U^{3'}p^{5'}U \xrightarrow{\text{NaOH}} pUp + Up + U\,(1 : 1 : 1)$$

$$pU^{2'}p^{5'}U^{3'}p^{5'}U \xrightarrow{\text{BAP}} U^{2'}p^{5'}U^{3'}p^{5'}U \xrightarrow{\text{N. P}_1} U^{2'}p^{5'}U + pU\,(1 : 1)$$

$$\downarrow \text{NaOH}$$

$$Up + U\,(2 : 1)$$

$$pU^{3'}p^{5'}U^{2'}p^{5'}U \xrightarrow{\text{N. P}_1} pU + pU^{2'}p^{5'}U\,(1 : 1)$$

$$pU^{3'}p^{5'}U^{2'}p^{5'}U \xrightarrow{\text{NaOH}} pUp + Up + U\,(1 : 1 : 1)$$

$$pU^{3'}p^{5'}U^{2'}p^{5'}U \xrightarrow{\text{BAP}} U^{3'}p^{5'}U^{2'}p^{5'}U \xrightarrow{\text{N. P}_1} U + pU^{2'}p^{5'}U\,(1 : 1)$$

$$\downarrow \text{NaOH}$$

$$Up + U\,(2 : 1)$$

The structures of three isomeric tetrauridylates containing one 3′-5′ linkage and two 2′-5′ linkages were determined by means of the following enzyme digestions.

$$pU^{2'}p^{5'}U^{3'}p^{5'}U^{2'}p^{5'}U \xrightarrow{\text{NaOH}} pUp + Up + U\,(1 : 2 : 1)$$

$$pU^{2'}p^{5'}U^{3'}p^{5'}U^{2'}p^{5'}U \xrightarrow{\text{BAP}} U^{2'}p^{5'}U^{3'}p^{5'}U^{2'}p^{5'}U$$

$$\downarrow \text{N. P}_1$$

$$U^{2'}p^{5'}U + pU^{2'}p^{5'}U\,(1 : 1)$$

$$pU^{3'}p^{5'}U^{2'}p^{5'}U^{2'}p^{5'}U \xrightarrow{NaOH} pUp + Up + U\,(1:2:1)$$

$$pU^{3'}p^{5'}U^{2'}p^{5'}U^{2'}p^{5'}U \xrightarrow{BAP} U^{3'}p^{5'}U^{2'}p^{5'}U_2{}'p^{5'}U \xrightarrow{N.\,P_1} U + pU^{2'}p^{5'}U^{2'}p^{5'}U\ (1:1)$$

$$pU^{2'}p^{5'}U^{2'}p^{5'}U^{3'}p^{5'}U \xrightarrow{NaOH} pUp + pU + U\,(1:2:1)$$

$$pU^{2'}p^{5'}U^{2'}p^{5'}U^{3'}p^{5'}U \xrightarrow{BAP} U^{2'}p^{5'}U^{2'}p^{5'}U^{3'}p^{5'}U \xrightarrow{N.\,P_1} U^{2'}p^{5'}U^{2'}p^{5'}U + pU\,(1:1)$$

Linkage isomers of pentauridylates were degraded by alkaline hydrolysis to yield pUp, Up and U in a nearly 1 : 3 : 1 ratio. Further characterization was not attempted.

Cyclic diuridylates were characterized by determination of their chromatographic mobilities and by means of the following enzyme digestions.

$$\boxed{\text{pUpU}} \xrightarrow{BAP} \not\rightarrow$$

$$\boxed{\text{pUpU}}\ (3'\text{-}5',\ 3'\text{-}5') \xrightarrow{N.\,P_1} pU$$

$$\boxed{\text{pUpU}}\ (3'\text{-}5',\ 2'\text{-}5') \xrightarrow{N.\,P_1} pU^{2'}p^{5'}U$$

$$\boxed{\text{pUpU}}\ (2'\text{-}5',\ 2'\text{-}5') \xrightarrow{N.\,P_1} \not\rightarrow$$

$$(2'\text{-}5',\ 2'\text{-}5') \xrightarrow{VPD} pU$$

The compounds $UppU^{2'}p^{5'}U$ and $UppU^{2'}p^{5'}U^{2'}p^{5'}U$ were identified by the following hydrolysis patterns.

$$UppU^{2'}p^{5'}U \xrightarrow{BAP} \not\rightarrow$$

$$UppU^{2'}p^{5'}U \xrightarrow{N.\,P_1} \not\rightarrow$$

$$UppU^{2'}p^{5'}U \xrightarrow{NaOH} UppUp + U\,(1:1) \xrightarrow{BAP} UppU$$

$$UppU^{2'}p^{5'}U^{2'}p^{5'}U \xrightarrow{BAP} \not\rightarrow$$

$$UppU^{2'}p^{5'}U^{2'}p^{5'}U \xrightarrow{N.\,P_1} \not\rightarrow$$

$$UppU^{2'}p^{5'}U^{2'}p^{5'}U \xrightarrow{NaOH} UppUp + Up + U\,(1:1:1) \xrightarrow{BAP} UppU$$

The products U, 3′,5′ cyclic UMP, pU and UppU were identified by paper chromatographic and HPLC comparisons with authentic samples. Authentic UppU and 3′,5′ cyclic UMP were prepared from pU using dicyclohexylcarbodiimide as a condensing agent according to the method of Khorana and his co-workers.[14,15]

References and Notes

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