LePecq, J. B., & Paoletti, C. (1976) *J. Mol. Biol. 27*, 87-106.
Lilley, D. M. J. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 6468-6472.
Mace, H. A. F., Pelham, H. R. B., & Travers, A. A. (1983) *Nature (London) 304*, 555-557.
Maniatis, T., Fritsch, E. F., & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
Maxam, A., & Gilbert, W. (1980) *Methods Enzymol. 65*, 499-560.
McCall, M., Brown, T., & Kennard, O. (1985) *J. Mol. Biol. 183*, 385-396.
Messing, J. (1983) *Methods Enzymol. 101*, 20-78.
Mirkovitch, J., Mirault, M., & Laemmli, U. K. (1984) *Cell (Cambridge, Mass.) 39*, 223-232.
Nickol, J. M., & Felsenfeld, G. (1983) *Cell (Cambridge, Mass.) 35*, 467-477.
Paulson, J. R., & Laemmli, U. K. (1977) *Cell (Cambridge, Mass.) 12*, 817-828.
Pulleyblank, D. E., Haniford, D. B., & Morgan, A. R. (1985) *Cell (Cambridge, Mass.) 42*, 271-280.
Schon, E., Evans, T., Welsh, J., & Efstradiatis, A. (1983) *Cell (Cambridge, Mass.) 35*, 837-848.
Selsing, E., Well, R. D., Alden, C. J., & Arnott, S. (1979) *J. Biol. Chem. 254*, 5417-5422.
Siebenlist, U., Henninghausen, L., Battey, J., & Leder, P. (1984) *Cell (Cambridge, Mass.) 37*, 381-391.
Sinden, R. R., Carlson, J. O., & Pettijohn, D. E. (1980) *Cell (Cambridge, Mass.) 21*, 773-783.
Sinden, R. R., Broyles, S. S., & Pettijohn, D. E. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 1797-1801.
Viera, J., & Messing, J. (1982) *Gene 19*, 259-268.
Vinograd, J., Lebowitz, J., & Watson, R. (1968) *J. Mol. Biol. 33*, 173-180.
Wang, J. C., & Hogan, M. (1985) *J. Biol. Chem. 260*, 8194-8202.
Wiegland, R. C., Beattie, K. L., Holloman, W. K., & Radding, C. M. (1977) *J. Mol. Biol. 116*, 805-824.

Respective Role of Each of the Purine N7 Nitrogens of 5′-*O*-Triphosphoadenylyl(2′→5′)adenylyl(2′→5′)adenosine in Binding to and Activation of the RNase L of Mouse Cells

Jean-Claude Jamoulle,[‡] Krystyna Lesiak,[§] and Paul F. Torrence*

Laboratory of Chemistry, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892

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ABSTRACT: Through a combination of chemical and enzymatic approaches a series of sequence-specific tubercidin-substituted ppp5′A2′p(5′A2′p)$_n$5′A (n = 1 to about 10; 2-5A) analogues were generated. In addition to the previously developed methodology of Imai and Torrence [Imai, J., & Torrence, P. F. (1985) *J. Org. Chem. 50*, 1418-1420], a new approach to synthesis of 2′,5′-linked oligonucleotides utilized adenosine in 3′,5′ linkage as a precursor to the targeted 5′-terminus of the desired product. For instance, A3′p5′A could be condensed under conditions of lead ion catalysis with tubercidin 5′-phosphate to give A3′p5′A2′p5′(c^{7}A). Treatment with the 3′,5′-specific nuclease P_1 led to p5′A2′p5′(c^{7}A). The combined use of the above procedures led to the synthesis of p5′(c^{7}A)2′p5′A2′p5′A, p5′A2′p5′(c^{7}A)2′p5′A, p5′A2′p5′A2′p5′(c^{7}A), and p5′A2p5′(c^{7}A)2′p5′(c^{7}A), which were converted to their corresponding 5′-triphosphates by the usual methods. Evaluation of these analogues for their ability to bind to and activate the 2-5A-dependent endonuclease (RNase L) of mouse L cells showed that there were small changes (≤10-fold) in the ability of the four tubercidin analogues to bind to RNase L. However, whenever the first and/or third adenosine nucleotide units were replaced by tubercidin, a dramatic decrease in ability to activate RNase L occurred. Only the second (from the 5′-terminus) adenosine residue could be replaced by tubercidin without any effect on RNase L activation ability.

2-5A[1] is a naturally occurring 2′,5′-linked oligonucleotide (Kerr & Brown, 1978) that is almost certainly involved in some of the antiviral effects of interferon [reviewed by Johnston and Torrence (1984)]. For instance, large amounts of 2-5A accumulate in interferon-treated encephalomyocarditis virus infected cells resulting in degradation of rRNA as well as poly(A+) RNA (Williams et al., 1979; Golgher et al., 1980; Knight et al., 1980). The complete 2-5A system currently includes the enzyme responsible for 2-5A generation or the 2-5A synthetase, the enzyme responsible for 2-5A action or the 2-5A-dependent endonuclease also known as RNase L, and a 2′,5′-phosphodiesterase that degrades 2-5A [reviewed by Johnston and Torrence (1984)]. The 2-5A system may also be involved in cell regulation and/or differentiation. For

[‡] Present address: Centre International de Recherches, Dermatologiques, Sophia Antipolis, Valbonne 06656, France.
[§] Present address: Center for Biomedical Research, University of Kansas, Lawrence, KS 66045.

[1] Abbreviations: 2-5A, ppp5′A2′p(5′A2′p)$_n$5′A, where n = 1 to about 10; 2-5A synthetase, the enzyme that, after activation by double-stranded RNA, effects the conversion nATP → pppA(pA)$_{n-1}$ + (n - 1)PP$_i$; PEI, poly(ethylenimine); HPLC, high-performance liquid chromatography; DMF, dimethylformamide; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride.

instance, it has been speculated that 2–5A could be involved in heterogeneous nuclear (hn) RNA processing (Nilsen et al., 1982). In addition, cellular levels of the 2–5A-dependent endonuclease are regulated during the differentiation of murine embryonal carcinoma cells (Krause et al., 1985). For these reasons, there has been interest in the possible utilization of the 2–5a system as a new approach to antiviral or antitumor agents (Torrence et al., 1984b). Specifically, we have been attempting to establish relationships between oligonucleotide structure and ability to bind to and activate the 2–5A-dependent endonuclease [reviewed by Torrence et al. (1986a,b)].

Recently, we reported the synthesis and biological activity of a tubercidin or 7-deazaadenosine analogue of 2–5A trimer in which all the adenosine residues were replaced by pyrrolopyrimidine nucleosides (Jamoulle et al., 1984), specifically, ppp5′(c^7A)$_2$′p5′(c^7A)2′p5′(c^7A). This analogue was bound to the 2–5A-dependent endonuclease of mouse L cells nearly as well as the parent 2–5A itself; however, this tubercidin analogue failed to activate the mouse RNase L even at concentrations 10^5 times that needed to cause endonuclease activation by 2–5A. These data suggested that one or more of the purine N7 residues of 2–5A may be involved in a protein–oligonucleotide binding interaction that must be completed before enzyme activation is possible. Other studies have indicated that the various individual residues of 2–5A have a differential importance in binding to and activation of the 2–5A-dependent endonuclease (Imai et al., 1985). This study was undertaken to ascertain the relative contribution of each of the three purine N7 moieties of 2–5A trimer and to determine if any correlation existed with previous studies of sequence-specific 2′,5′-oligonucleotides as RNase L activators.

Materials and Methods

The sources of the various chemicals, biochemicals, and enzymes used herein have been described previously (Jamoulle et al., 1984; Torrence et al., 1984a; Imai & Torrence, 1985; Lesiak et al., 1983).

Thin-layer chromatography (TLC) was either on silica gel GF plates with system A (2-propanol/H_2O/concentrated NH_4OH, 7:2:1) or on PEI–cellulose plates with system B (0.1 M NH_4HCO_3). HPLC was carrier out on either (I) a Model 110 solvent delivery system and a Model 421 controller (Beckmann, Berkeley, CA) with an ISCO (Lincoln, NB) UA-5 detector and an Altex (Berkeley, CA) Model C-RIA integrator or (II) a Model 112 solvent delivery system (Beckmann) with a Model 420 controller and a Model 153 analytical UV detector (Beckmann) and a Hewlett-Packard 3390A integrator. HPLC column/solvent systems were as follows: system 1, Ultrasphere ODS/RP 4.6 × 150 mm column with a 0–40% B gradient in 25 min at a flow rate of 1 mL/min; system 2, above column and gradient but a 1.5 mL/min flow rate; system 3a, above column and gradient but a 0.75 mL/min flow rate; system 3b, same column but 0–50% B in 25 min with a 0.75 mL/min flow rate; system 3c, Ultrasphere ODS/RP 4.6 × 250 mm column with a 0–40% B gradient in 25 min and a flow rate of 1 mL/min; system 4, Zorbax ODS/RP 9.4 × 250 mm with a 0–50% B gradient in 25 min and a flow rate of 2.7 mL/min; system 5, same column but a 0–40% B gradient in 28 min and a flow rate of 1.5 mL/min; system 6a, Zorbax ODS/RP 21.2 × 250 mm column with a 0–50% B gradient in 25 min and a 7.5 mL/min flow rate; system 6b, same column and gradient but a flow rate of 2.75 mL/min for 2 min then 7.5 mL/min; system 7, Bondapak ODS/RP 3.9 × 300 mm column with a 0–40% B gradient in 25 min and a 1.0 mL/min flow rate; system 8, Bondapak ODS/RP 7.6 × 300 mm column with a 0–50% B gradient in 25 min at 2 mL/min flow rate. In all the above systems, solvent A was 0.05 M ammonium phosphate (pH 7.0), and solvent B was methanol/H_2O, 1:1.

Proton NMR were recorded on a Varian HR 220-MHz instrument with D_2O as solvent and acetone as an internal standard. Each sample was repeatedly evaporated with D_2O and treated with Chelex 100 (ammonium form) before recording the spectrum. ^{31}P NMR spectra were obtained on a Varian instrument operating at 109 MHz and chemical shifts reported relative to an external 0.85% H_3PO_4 standard.

When reference is made to an HPLC separation, the instrumentation will be given first, followed by the specific column/solvent program employed. For instance, I.1 refers to the 110 solvent delivery system/421 controller/ISCO detector/C-RIA integrator using an Ultrasphere ODS/RP 4.6 × 150 mm column with a 0–40% B gradient in 25 min at a flow rate of 1 mL/min.

General Procedure for Preparation of 5′-Phosphoramidates. As an example, 7-deazaadenylyl(2′→5′)adenosine 5′-monophosphate [p5′(c^7A)2′p5′A, 140 A_{262} units, 6.5 μmol] was dissolved in dry Me_2SO (500 μL) containing imidazole (19.25 mg, 28 μmol), triphenylphosphine (28.9 mg, 11 μmol), triethylamine (1 μL), and dipyridyl disulfide (24.7 mg, 11 μmol). The reaction mixture was monitored by TLC on silica gel with solvent system A. After 40 min of stirring at room temperature, the yellow solution was transferred dropwise to a stirred solution of sodium iodide (50 mg) in dry acetone (10 mL), and the precipitate was collected by centrifugation and washed 3 times with acetone (1 mL each time). The resulting sodium salt of the 5′-phosphoroimidazolidate of (c^7A)2′p5′A was dried 3 h in vacuo. The material was homogeneous as judged by TLC and HPLC in system A and system II (solvent system 2), respectively. Other phosphoroimidazolidates and phosphoromorpholidates were generated in a similar manner.

Synthesis of Sequence-Specific Adenylate and 7-Deazaadenylate Containing Cooligonucleotides p5′A2′p5′-(c^7A)2′p5′(c^7A), p5′A2′p5′A2′p5′(c^7A), p5′(c^7A)2′p5′A2′p5′A, and p5′A2′p5′(c^7A)2′p5′A. (1) Preparation of A3′p5′A2′p5′(c^7A)2′p5′(c^7A). The dinucleotide A3′p5′A (1140 A_{258} units, 42 μmol, ammonium salt) was dissolved in 1.2 mL of *N*-methylimidazole buffer (0.2 M, pH 7.2) at 0 °C. Freshly prepared imidazolidate of p5′(c^7A)2′p5′(c^7A) (500 A_{270} units, 22.5 μmol, sodium salt) and 0.1 mL of lead nitrate (0.25 M) consecutively were added to the above dinucleotide. The progress of the reaction was monitored by HPLC with system I and solvent system 3a. The reaction mixture was stirred at 4 °C for 2 days until the reaction was complete. Chelex 100 (2-mL bed volume) then was added to the yellowish reaction, and the entire mixture was stirred 30 min at 4 °C to remove all Pb^{2+} ions. The Chelex was filtered off and washed with water (1 mL), and the crude reaction mixture and washes were purified by HPLC (system I, solvent 5).

Fractions containing the tetramer core A3′p5′A2′p5′-(c^7A)2′p5′(c^7A) were pooled and evaporated to dryness in vacuo at 30 °C, and the resulting residue was applied to a DEAE-Sephadex A-25 column to remove phosphate of the HPLC buffer. Elution was accomplished with a linear gradient of 0–0.3 M triethylammonium bicarbonate (pH 7.6). Fractions of tetramer core (60 A_{262} units) were pooled and evaporated to dryness in vacuo at 30 °C. The residue was taken up in water, and the water removed in vacuo. This process was repeated several times to ensure decomposition of all the triethylammonium bicarbonate. Likewise, from the same reaction the corresponding 3′,5′-sequence isomer A3′p5′A3′p5′(c^7A)2′p5′(c^7A) (35 A_{262} units) could be isolated.

(2) *Preparation of p5′A2′p5′(c^7A)2′p5′(c^7A) by Nuclease P_1 Cleavage of A3′p5′A2′p5′(c^7A)2′p5′(c^7A).* Pure tetramer A3′p5′A2′p5′(c^7A)2′p5′(c^7A) (60 A_{262} units, 51 μmol, triethylammonium salt) was dissolved in a buffer containing 2-(*N*-morpholino)ethanesulfonic acid (50 mM, pH 6.0), EDTA (1 mM), and nuclease P_1 (5 μL, 2 mg/mL), and the entire mixture was incubated overnight at 37 °C. The incubation mixture was then heated to 100 °C for 4 min, and the denatured insoluble protein was removed, after cooling, by centrifugation at 10000*g*. The supernatant was applied to a DEAE-Sephadex A-25 (HCO_3^-) column. Elution was accomplished with a linear gradient from 0.0 to 0.4 M triethylammonium bicarbonate (pH 7.5). Fractions containing the trimer monophosphate were pooled and evaporated to dryness in vacuo at 40 °C as described previously.

(3) *Procedure for Preparation of p5′A2′p5′A2′p5′(c^7A).* The ammonium salt of A2′p5′A (1032 A_{258} units, 25 mg, 40 μmol) and the freshly prepared imidazolidate of p5′(c^7A) (235 A_{270} units, 10 mg, 23 μmol, sodium salt) were mixed in *N*-methylimidazole buffer (2.2 mL, pH 7.2) at 0 °C, and 125 mL of $PbNO_3)_2$ (0.25 M) was added. The disappearance of the phosphoroimidazolidate of tubercidin was determined by HPLC (system II using solvent system 4). When the reaction was complete (~2 days), Chelex (2 mL) was added to the mixture and the suspension was stirred until clarification of the supernatant occurred. The pH of the filtrate and washings was adjusted to 7.0, and the trimer core product A2′p5′A2′p5′(c^7A) was separated by HPLC with system II and solvent 6a. Appropriate fractions were pooled (retention time ~28.9 min), and solvent was removed in vacuo. The residue was taken up in 1.5 mL f H_2O and applied to the same column and eluted with pure water to remove phosphate ions. This was then placed on a DEAE-Sephadex A-25 column that was eluted with a gradient of 0.0–0.22 M triethylammonium bicarbonate (250 mL vs. 250 mL). Fractions containing the oligomer were pooled and evaporated to dryness to give pure A2′p5′A2′p5′(c^7A) (72 A_{262} units, 2.3 μmol). The enzymatic phosphorylation of A2′p5′A2′p5′(c^7A) to give p5′A2′p5′A2′p5′(c^7A) was carried out according to the procedures described elsewhere (Torrence et al., 1986a,b).

(4) *Procedure for Preparation of p5′(c^7A)2′p5′A2′p5′A.* The sodium salt of the morpholidate of tubercidin 5′-phosphate (25 mg, 560 A_{270} units, 55 μmol) and the 5′-phosphoroimidazolidate of A2′p5′A (25.6 mg, 680 A_{258} units, 27.2 μmol) were mixed in a solution of 0.2 M imidazole nitrate buffer (pH 7.8, 1 mL), and $Pb(NO_3)_2$ (35 μL of 0.25 M) was added. After completion of the reaction as indicated by HPLC with system II and solvent system 3, Chelex 100 was added and the mixture stirred at 0 °C until the supernatant was clear. The Chelex was removed by filtration and washed with water, and the crude reaction mixture was purified by column chromatography on a DEAE-Sephadex A-25 column (1 × 25 cm, elution with a linear gradient of 0.0–0.3 M triethylammonium bicarbonate, 500 mL + 500 mL). Fractions 105–119 contained the product, namely, the morpholidate of p5′(c^7A)2′p5′A2′p5′A. Later fractions (131–145) contained the sequence isomer [morpholidate of p5′(c^7A)3′p5′A2′p5′A]. Fractions containing the desired 2′,5′ isomer were combined, and solvent was removed in vacuo. Triethylammonium bicarbonate was removed by addition and evaporation of water in vacuo. The product was dissolved in ammonium phosphate buffer (50 mM, pH 4.2), and incubation was at 45 °C for 6 h. The cleavage of the morpholine moiety was monitored by HPLC (system II, solvent system 3b). The resulting p5′(c^7A)2′p5′A2′p5′A was purified by DEAE-column chromatography and isolated in the usual manner as the triethylammonium salt.

(5) *Synthesis of p5′A2′p5′(c^7A)2′p5′A.* For this oligonucleotide, the basic approach employed earlier (Imai & Torrence, 1985) was employed. Thus, the morpholidate of tubercidin 5′-monophosphate (836 A_{270} units, 82 μmol) was reacted with the 5′-phosphoroimidazolidate of adenosine (631 A_{257} units, 41 μmol) in 1.0 mL of *N*-methylimidazole buffer (pH 7.1) with 54 μL of $Pb(NO_3)_2$ (0.25 M) at 4 °C. After Chelex deionization, HPLC (system II, solvent system 3b), and DEAE-column chromatography, the morpholidate of p5′(c^7A)2′p5′A (344 A_{262} units, 16 μmol) was obtained. The morpholidate moiety was removed by acidic cleavage at pH 4.3 (7 h at 43 °C) to give, after HPLC (system II, solvent system 3b), a quantitative yield of the free monophosphate p5′(c^7A)2′p5′A. A portion of this was converted to the imidazolidate in the usual way, and then 6.5 μmol of this 5′-phosphoroimidazolidate of (c^7A)2′p5′A was reacted with adenosine 5′-phosphoromorpholidate (345 A_{258} units, 22.7 μmol) in 0.8 mL of *N*-methylimidazole buffer (pH 7.1) containing 7 μL of $Pb(NO_3)_2$ (0.25 M). After the usual workup, including Chelex treatment, HPLC (system II, solvent system 3c), and DEAD-Sephadex chromatography, the morpholidate of p5′A2′p5′(c^7A)2′p5′A was obtained (25 A_{261} units, 0.8 μmol). This could either by hydrolyzed to the monophosphate or converted to the corresponding 5′-triphosphate (vide infra).

Preparation of Oligonucleotide 5′-Triphosphates. As an example, the imidazolidate of p5′A2′p5′(c^7A)2′p5′(c^7A) (35 A_{263} units, 1.2 μmol), generated by the above procedures and dried 3 h in vacuo, was dissolved in DMF (200 μL), and tri-*n*-butylammonium pyrophosphate in DMF (0.5 M solution, 108 μL, 54 μmol) was added. The solution was stoppered and kept in a desiccator at ambient temperature for 24 h. The DMF then was evaporated in vacuo, and the residue was diluted with triethylammonium bicarbonate buffer (500 μL, 0.25 M). The pH of this solution was adjusted to 7.5, and the entire mixture was applied to a DEAE-Sephadex A-25 (HCO_3^-) column (1 × 20 cm). The column was eluted with a linear gradient of 0.0–0.7 M (250 mL + 250 mL) triethylammonium bicarbonate. The product ppp5′A2′p5′(c^7A)2′p5′(c^7A) was obtained as the triethylammonium salt in a 73% yield (26 A_{263} units, 0.8 μmol) on the basis of starting monophosphate. The identical procedure could be applied to 5′-phosphoromorpholidate as obtained from a typical lead ion catalyzed coupling reaction. Yields of triphosphate varied from 38% to 92% with the usual being about 70%.

Characterization of Oligonucleotides. The purity of each oligomeric product was checked on TLC (system A) and by HPLC (system II, solvent system 1; system I, solvent system 7). Each oligomer was tested for resistance to the action of nuclease P_1 [40 μg/mL in a buffer of 2-(*N*-morpholino)-ethanesulfonic acid, 50 mM, pH 6). The conditions of digestion and products of digestion with bacterial alkaline phosphatase or snake venom phosphodiesterase are given in Table I. Proton and phosphorus NMR characteristics are listed in Tables II and III. Specific HPLC retention times for compounds of interest are given in the following manner; the compound is listed followed by its retention time on HPLC system II with solvent system 1 and then followed by its retention time on HPLC system II with solvent system 7. ND means the value was not determined. The oligomers and their respective retention times are as follows: ppp5′A2′p5′A2′p5′A, 8.5, 10.4; p5′A2′p5′A2′p5′A, 10.0, 14.7; ppp5′(c^7A)2′p5′(c^7A)2′p5′(c^7A), 8.0, 7.4; p5′(c^7A)2′p5′(c^7A)2′p5′(c^7A), 8.7, 12.4; ppp5′A2′p5′(c^7A)2′p5′(c^7A), 7.9, 7.5; p5′(c^7A)2′p5′(c^7A),

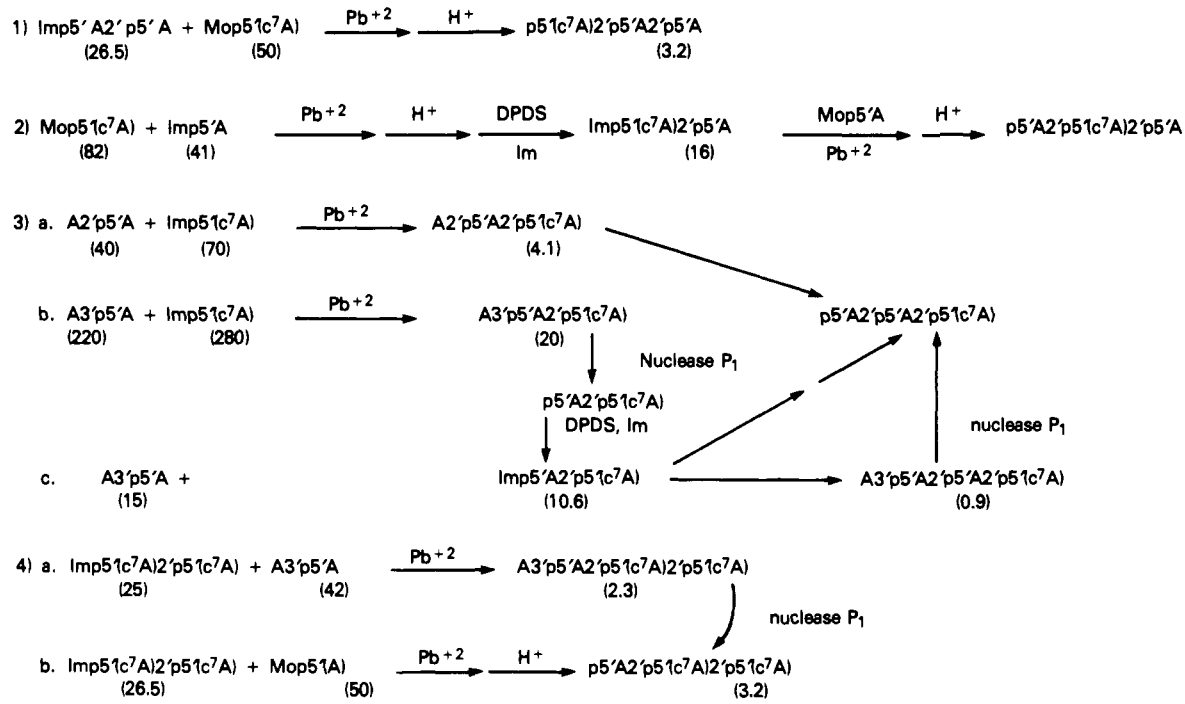

FIGURE 1: Synthetic schemes to adenosine and tubercidin sequence isomers and analogues of 2–5A. Abbreviations: Im, imidazole; Mo, morpholine; DPDS, dipyridyl disulfide. Typical reactant quantities and product yields (in μmol) are indicated in parentheses for several reactions.

7.0, 8.5; p5′A2′p5′(c⁷A)2′p5′(c⁷A), 8.4, 11.9; A2′p5′(c⁷A)-2′p5′(c⁷A), 15.6, ND; ppp5′A2′p5′A2′p5′(c⁷A), 8.6, 5.6; p5′A2′p5′(c⁷A), 7.4, 8.2; p5′A2′p5′A2′p5′(c⁷A), 5.2, 13.6; A2′p5′A2′p5′(c⁷A), 15.4, ND; ppp5′(c⁷A)2′p5′A2′p5′A, 8.2, 10.8; p5′(c⁷A)2′p5′A, 6.5, 7.7; p5′(c⁷A)2′p5′A2′p5′A, 8.8, 13.2; (c⁷A)2′p5′A2′p5′A, 21.1, ND; ppp5′A2′p5′(c⁷A)2′p5′A, 7.3, 7.2; p5′A2′p5′(c⁷A)2′p5′A, 8.4, 12.3; A2′p5′(c⁷A)2′p5′A, 19.1, ND.

Biological Activity Studies. The preparation of mouse L cell extracts, encephalomyocarditis virus RNA, and the technique and conditions for cell-free protein synthesis were as described earlier (Torrence & Friedman, 1979). Radiobinding assays with ppp5′A2′p5′A2′p5′A2′p5′A3′[^{32}P]p5′C3′p of specific activity-3000 Ci/mmol (Amersham, Chicago, IL) were according to Knight et al. (1980), and ribosomal RNA cleavage assays were according to Wreschner et al. (1981).

Results

Chemistry. Several different approaches were used to prepare the tubercidin sequence-specific analogues of 2–5A (summarized in Figure 1). We previously reported (Imai & Torrence, 1985) an extension of the lead ion catalyzed polymerization of nucleoside 5′-phosphoroimidazolidates (Sawai & Ohno, 1981ab; Sawai et al., 1979). This permits the synthesis of sequence-specific 2′,5′-oligonucleotides of the general formula p5′X2′p5′Y2′p5′Z. The crux of this method is the use of the 5′-phosphoromorpholidate moiety as a protecting group of the nucleoside destined to become the 5′-terminal residue. This morpholidate group is unreactive under the conditions of Pb^{2+}-catalyzed ligation, but it can be removed readily with mild acid treatment to regenerate the corresponding 5′-monophosphate and can, if required, be converted to the corresponding 5′-triphosphate.

During the time this latter methodology was under development we were engaged in a separate but parallel synthetic approach using a different protecting group for the targeted 5′-terminal nucleotide. In this scheme, the nucleoside destined to become the 5′-terminus is introduced as the 3′-terminal residue of a 3′,5′-linked dinucleotide, for instance, as A3′p5′A. After conjugation in 2′,5′ linkage to a nucleotide or another oligonucleotide, digestion with the 3′,5′-specific nuclease P_1 can be employed to cleave the residue in 3′,5′ linkage. This results in a 2′,5′-oligomer with a 5′-phosphate terminus. This latter methodology is illustrated in Figure 1 for the synthesis of p5′A2′p5′(c⁷A) (scheme 3b) p5′A2′p5′A2′p5′(c⁷A) (scheme 3c), and p5′A2′p5′(c⁷A)2′p5′(c⁷A) (scheme 4a). Like the morpholidate approach described above, this latter approach is useful because it provides a relatively quick access to sequence-specific oligomers even though yields are not high. It is limited in that the nucleotide destined to become the 5′-residue in the 2′,5′-linked oligomer must be available (or able to be synthesized) as the 3′-terminus of a 3′,5′-linked dinucleotide. In addition, it generates significant quantities of 3′,5′ isomer in the Pb^{2+}-catalyzed coupling, usually $^1/_2$–$^1/_3$ as much as the amount of 2′,5′-linkage.

Because of the ready commmercial availability of A3′p5′A, it was convenient to prepare both p5′A2′p5′A2′p5′(c⁷A) and p5′A2′p5′(c⁷A)2′p5′(c⁷A) by this latter approach. Thus, reaction of Imp5′(c⁷A)2′p5′(c⁷A) with A3′p5′A followed by nuclease P_1 cleavage gave p5′A2′p5′(c⁷A)2′p5′(c⁷A), which was also generated by reaction of Imp5′(c⁷A)2′p5′(c⁷A) with Mop5′(c⁷A) and subsequent acid hydrolysis (Figure 1). The products of the two different syntheses were identical as judged by both HPLC and TLC. Three different pathways were used to prepare p5′A2′p5′A2′p5′(c⁷A) (Figure 1). The dimer A3′p5′A was ligated to Imp5′(c⁷A) to give, after nuclease P_1 digestion, p5′A2′p5′(c⁷A), which after conversion to the imidazolidate, Imp5′A2′p5′(c⁷A), could be condensed either with A3′p5′A or with Mop5′A to yield p5′A2′p5′A2′p5′(c⁷A) after either nuclease P_1 treatment or mild acid hydrolysis, respectively. Finally, the dimer A2′p5′A could be extended with Imp5′(c⁷A) to give the core trimer A2′p5′A2′p5′(c⁷A), which could be phosphorylated with T4 polynucleotide kinase and ATP to produce material identical by HPLC and TLC with the p5′A2′p5′A2′p5′(c⁷A) generated by the alternative sequence (Figure 1, schemes 3b and 3c).

The remaining two analogues were made by the previously described approach (Imai & Torrence, 1985). Thus in one

Table I: Enzymatic Characterization of Oligonucleotides

oligomer	alkaline phosphatase digest, product + retention time[a]	venom phosphodiesterase digest, products + retention time[b]
p5′A2′p5′(c^{7}A)2′p5′(c^{7}A)	A2′p5′(c^{7}A)2′p5(c^{7}A) (19.63)	1 p5′A (6.3) + 2 p5′(c^{7}A) (5.7)
p5′A2′p5′A2′p5′(c^{7}A)	A2′p5′A2′p5′(c^{7}A) (19.35)	2 p5′A (6.5) + 1 p5′(c^{7}A) (5.6)
p5′(c^{7}A)2′p5′A2′p5′A	(c^{7}A)2′p5′A2′p5′A (21.12)	2 p5′A (6.4) + 1 p5′(c^{7}A) (5.4)
p5′A2′p5′(c^{7}A)2′p5′A	A2′p5′(c^{7}A)2′p5′A (19.14)	2 p5′A (6.0) + 1 p5′(c^{7}A) (5.1)

[a] Conditions: 3 A_{260} units of substrate and 0.01 unit of bacterial alkaline phosphatase in 20 μL of 0.1 M Tris-HCl (pH 8.5) at 37 °C for 2.5 h. Analysis was with HPLC system II with solvent system 3a. HPLC retention times are given in parentheses. [b] Conditions: 3 A_{260} units of substrate and 0.01 unit of snake venom phosphodiesterase in 20 μL of 0.05 M Tris–acetate (pH 8.8) and 0.005 M $MgCl_2$ at 37 °C for 2.5 h. Analysis was with HPLC system II with solvent system 3c. HPLC retention times are given in prentheses. Product molar ratios were determined with the aid of HPLC integrations and the molar extinction coefficients of the products.

Table II: Characteristic Proton NMR Signals of Synthetic Oligonucleotides[a]

oligomer	aromatic ring protons C2 and C7 and/or purine C8	pyrrolopyrimidine protons C8 and anomeric protons C1
p5′A2′p5′(c^{7}A)	7.98 (1 H, s), 7.78 (1 H, s), 7.64 (1 H, s), 6.94 (1 H, d)	5.96 (1 H, d), 5.91 (1 H, d), 5.74 (1 H, d)
p5′(c^{7}A)2′p5′A	8.01 (1 H, s), 7.82 (2 H, s), 7.20 (1 H, d)	6.20 (1 H, d), 6.15 (1 H, d), 5.66 (1 H, d)
p5′(c^{7}A)2′p5′A2′p5′[b]	8.00 (1 H, s), 7.79 (1 H, s), 7.74 (1 H, s), 7.65 (2 H, s), 6.84 (1 H, d)	6.07 (1 H, d), 6.02 (1 H, s), 5.70 (1 H, d), 5.63 (1 H, d)
p5′A2′p5′(c^{7}A)2′p5′(c^{7}A)[b]	7.94 (1 H, s), 7.89 (1 H, s), 7.70 (1 H, s), 7.68 (1 H, s)	6.88 (1 H, d), 6.83 (1 H, d), 6.04 (1 H, d), 5.91 (2 H, s), 5.73 (1 H, d), 5.66 (1 H, d)
p5′A2′p5′(c^{7}A)2′p5′A[b]	8.02 (1 H, s), 7.89 (1 H, s), 7.70 (1 H, s), 7.68 (1 H, s), 7.66 (1 H, s), 6.90 (1 H, d)	5.93 (1 H, s), 5.89 (1 H, s), 5.73 (1 H, d), 5.59 (1 H, d)
p5′A2′p5′A2′p5′(c^{7}A)[b]	7.90 (1 H, s), 7.75 (1 H, s), 7.71–7.68 (3 H, m), 6.93 (1 H, d)	6.09 (1 H, d), 5.87 (1 H, s), 5.75 (1 H, s), 5.72 (1 H, d)
A2′p5′A2′p5′(c^{7}A)	7.94 (1 H, s), 7.89 (1 H, s), 7.76 (1 H, s), 7.69 (1 H, s), 7.55 (1 H, s), 6.90 (1 H, d)	6.10 (1 H, d), 5.87 (1 H, d), 5.74 (1 H, d), 5.69 (1 H, d)
ppp5′A2′p5′(c^{7}A)2′p5′(c^{7}A)	8.00 (1 H, s), 7.88 (1 H, s), 7.73 (1 H, s), 7.71 (1 H, s), 6.98 (1 H, d)	6.15 (1 H, d), 5.81 (3 H, m), 5.75 (1 H, d)
ppp5′A2′p5′A2′p5′(c^{7}A)	8.03 (1 H, s), 7.75 (1 H, s), 7.52 (2 H, s), 7.36 (1 H, s), 7.04 (1 H, d)	6.30 (1 H, d), 5.89 (1 H, d), 5.66 (2 H, m)
ppp5′(c^{7}A)2′p5′A2′p5′A	8.02 (1 H, s), 7.85 (1 H, s), 7.82 (1 H, s), 7.79 (1 H, s), 7.73 (1 H, s), 7.15 (1 H, d)	6.28 (1 H, d), 6.01 (1 H, d), 5.75 (1 H, d), 5.66 (1 H, d)
ppp5′A2′p5′(c^{7}A)2′p5′A	8.03 (1 H, s), 7.89 (1 H, s), 7.76 (3 H, m), 6.89 (1 H, d)	5.90 (1 H, d), 5.89 (1 H, d), 5.80 (1 H, d), 5.63 (1 H, d)

[a] Conditions are as described under Materials and Methods. x = singlets; d = doublets; m = multiplets. Coupling constants are not given but ranged from 2 to 6 hZ. [b] As morpholidate derivatives.

Table III: Comparison of ^{31}P NMR Chemical Shifts of 2′,5′-Linked Adenylate and 7-Deazaadenylate Cooligonucleotides[a]

oligomer	internucleotide phosphates	5′-phosphates		
		α	β	α
ppp5′A2′p5′A2′p5′A	−1.06, −1.38	−11.38	−22.52	−6.84
ppp5′(c^{7}A)2′p5′(c^{7}A)2′p5′(c^{7}A)	−0.96, −1.10	−11.08	−22.10	−8.71
ppp5′A2′p5′(c^{7}A)2′p5′(c^{7}A)	−3.00, −3.27	−13.24	−24.46	−11.03
ppp5′A2′p5′A2′p5′(c^{7}A)	−3.39, −3.39	−13.48	−25.00	−12.38
ppp5′(c^{7}A)2′p5′A2′p5′A	−3.00, −3.30	−13.56	−25.08	−12.52
ppp5′A2′p5′(c^{7}A)2′p5′A	−3.12, −3.12	−13.37	−24.98	−12.38

[a] See Materials and Methods for details.

case, Imp5′A2′p5′A was ligated to Mop5′(c^{7}A) to give after hydrolysis p5′(c^{7}A)2′p5′A2′p5′A. Finally, the dimer p5′-(c^{7}A)2′p5′A was synthesized by condensation of Mop5′(c^{7}A) with Imp5′A. Then, after conversion to the corresponding imidazolidate, it was joined to Mop5′A to give, following acid treatment, p5′A2′p5′(c^{7}A)2′p5′A (Figure 1, scheme 2).

All of the requisite trinucleoside 5′-monophosphates could be converted to the corresponding 5′-triphosphates by standard techniques without any complications.

In addition to the fact that two or three different synthetic approaches were employed to generate p5′A2′p5′(c^{7}A)2′p5′-(c^{7}A) and p5′A2′p5′A2′p5′(c^{7}A), respectively, the following spectral and enzymic digest data substantiated the assigned oligonucleotide structures.

(1) Digestion of each product with alkaline phosphatase effected an increased retention time that was in accord with a 5′-unphosphorylated trinucleotide (Table I).

(2) Venon phosphodiesterase digestion gave the expected nucleotide products in the expected ratio (Table I).

(3) The proton magnetic resonance spectra of the various synthetic products were in accord with their assigned structures. Typically, the requisite number of characteristic anomeric protons and base (either purine and/or pyrrolopyrimidine) protons were located (Table II).

(4) For each triphosphate, the appropriate number of and multiplicity for the internucleotide phosphorus atoms and the α, β, and γ-phosphorus atoms of the 5′-triphosphate moiety could be discerned (Table III).

Biological Activities. The ability of the tubercidin analogues to bind to the 2–5A-dependent endonuclease was determined by the radiobinding assay described by Knight et al. (1980) (Figure 2). All of the sequence-specific oligomers were bound effectively to the endonuclease and could be arranged in the following order of decreasing activity (IC_{50} values in parentheses): ppp5′A2′p5′A2′p5′A (3×10^{-10} M) > ppp5′A2′p5′(c^{7}A)2′p5′A (5×10^{-10} M) > ppp5′(c^{7}A)-2′p5′A2′p5′A (1×10^{-9} M) > ppp5′A2′p5′A2′p5′(c^{7}A) (6×10^{-9} M). These results differ slightly from an earlier study

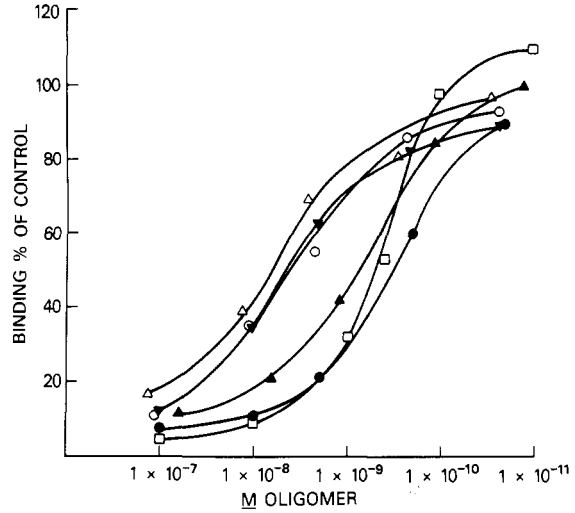

FIGURE 2: Ability of various tubercidin–adenosine sequence isomers to bind to the 2–5A-dependent endonuclease (RNase L) as determined by the method of Knight et al. (1980): (○) ppp5′A2′p5′(c⁷A)-2′p5′(c⁷A); (△) ppp5′A2′p5′A2′p5′(c⁷A); (▲) ppp5′(c⁷A)2′p5′A2′p5′A; (□) ppp5′A2′p5′(c⁷A)2′p5′A; (▼) ppp5′(c⁷A)2′p5′(c⁷A)2′p5′(c⁷A); (●) ppp5′A2′p5′A2′p5′A.

(Jamoulle et al., 1984) that compared 2–5A with the all-tubercidin-substituted analogues ppp5′(c⁷A)2′p5′(c⁷A)2′p5′-(C⁷A). In the latter instance, the tubercidin analogue was bound only half as effectively as 2–5A itself.

The ability of each of the analogues to inhibit protein synthesis in a cell-free system derived from mouse L cells and programmed with encephalomyocarditis virus RNA was determined as a measure of their ability to activate RNase L. These experiments were performed several different times in several different formats; representative results are pictured in Figure 3. All of the analogues were capable of inhibiting translation, albeit at significantly different concentrations and to significantly different extents than 2–5A trimer triphosphate. As shown before (Jamoulle et al., 1984), ppp5′-(c⁷A)2′p5′(c⁷A)2′p5′(c⁷A) did not inhibit protein synthesis in the L cell system up to concentrations of 10^{-5} M. Somewhat more effective were the analogues that bore a 7-deazaadenosine substitution only at the 2′-terminus, including ppp5′A2′p5′A2′p5′(c⁷A) and ppp5′A2′p5′(c⁷A)2′p5′(c⁷A). These latter two oligomers did not cause the same degree of inhibition of protein synthesis as did 2–5A itself. The greatest extent of inhibition of translation with these latter analogues was about 22%. The concentration for effecting a half-maximal response was approximately 2×10^{-8} M. The oligonucleotide bearing tubercidin at only the 5′-terminus was a more potent inhibitor of translation, albeit still not as potent as 2–5A. The oligomer ppp5′(c⁷A)2′p5′A2′p5′A also could not effect the same degree of translational inhibition as could 2–5A, although it was more effective in this regard than either ppp5′A2′p5′A2′p5′(c⁷A) or ppp5′A2′p5′(c⁷A)2′p5′(c⁷A). Thus, ppp5′(c⁷A)2′p5′A2′p5′A brought about a 32–34% inhibition of protein synthesis, and the concentration necessary to achieve a half-maximal effect was approximately 4×10^{-9} M. Under these conditions, ppp5′A2′p5′A2′p5′A caused a 62–80% inhibition of translation, and the concentration required to effect a half-maximal response was 6×10^{-10} to 1.5×10^{-9} M, in agreement with many previously reported experiments. Finally, the oligomer ppp5′A2′p5′(c⁷A)2′p5′A was just as effective as 2–5A itself both in regard to the extent of inhibition of translation it could achieve and in regard to the concentration needed to bring about a half-maximal response. In Figure 3b, ppp5′A2′p5′(c⁷A)2′p5′A appears somewhat more

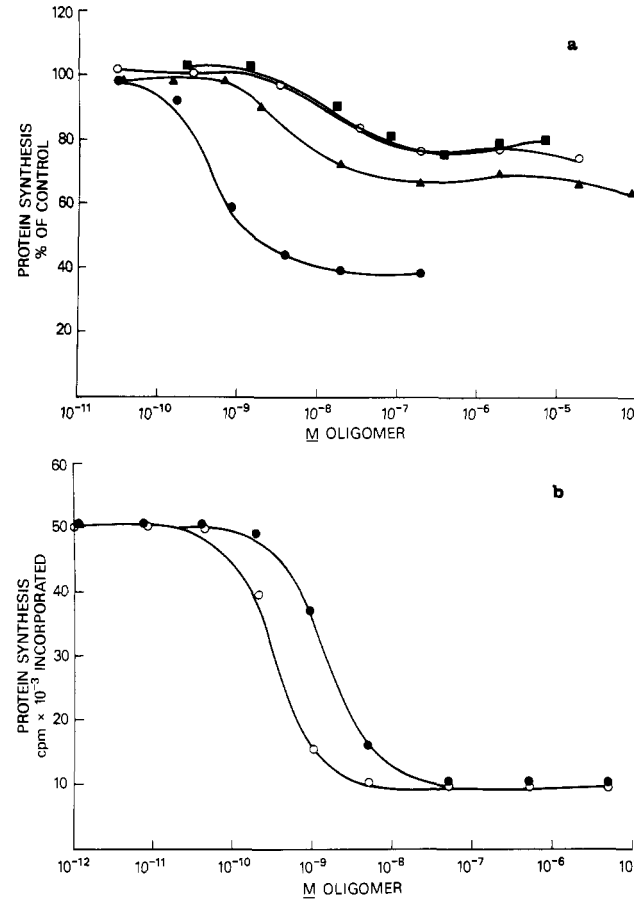

FIGURE 3: Protein synthesis inhibition by 2–5A and various tubercidin analogs. (a) (●) ppp5′A2′p5′A2′p5′A; (▲) ppp5′(c⁷A)2′p5′A2′p5′A; (○) ppp5′A2′p5′(c⁷A)2′p5′(c⁷A); (■) ppp5′A2′p5′A2′p5′(c⁷A). (b) (●) ppp5′A2′p5′A2′p5′A; (○) ppp5′A2′p5′(c⁷A)2′p5′A.

effective than ppp5′A2′p5′A2′p5′A, but the difference in IC_{50}'s (1.5×10^{-9} vs. 5×10^{-10} M) was within the limits of error of the assay system.

The ability of the various tubercidin analogues to bring about ribosomal RNA cleavage (Wreschner et al., 1981) was employed as an independent approach to ascertain their ability to activate RNase L (Figure 4). In these experiments, L cell extracts were incubated under protein synthesis conditions (but without EMCV RNA, labeled amino acid, or energy mix) with or without 2–5A or an analogue, and then rRNA was isolated by phenol treatment and ethanol precipitation, and the RNA was separated by gel electrophoresis. As has been described previously (Wreschner et al., 1981), ppp5′A2′p5′A2′p5′A, at concentrations as low as 10^{-9} M, could effect cleavage of 28S and 18S RNA into several discrete bands of lower molecular weight in a pattern typical for 2–5A-induced degradation (Figure 4a, tracks 3–5) in agreement with ability to inhibit translation. Under the identical conditions, neither ppp5′A2′p5′(c⁷A)2′p5′(C⁷A) (Figure 4a, tracks 8–11) nor ppp5′A2′p5′A2′p5′(c⁷A) (Figure 4a, tracks 16–18) could induce cleavage even at 2×10^{-6} M. Somewhat more effective, in general agreement with the cell-free translation results, was ppp5′(c⁷A)2′p5′A2′p5′A, which could effect RNA breakdown at 2×10^{-6} M (Figure 4a, tracks 12–15). Finally, also in good agreement with the protein synthesis inhibition data of Figure 3, ppp5′A2′p5′(c⁷A)2′p5′A was just as effective as 2–5A itself at inducing rRNA degradation (Figure 4b, tracks 1–6).

A comparative study was performed on the rate of degradation of the various tubercidin analogues compared to 2–5A under conditions of the radiobinding assay. An initial concentration of 2×10^{-5} M oligomer was employed, and con-

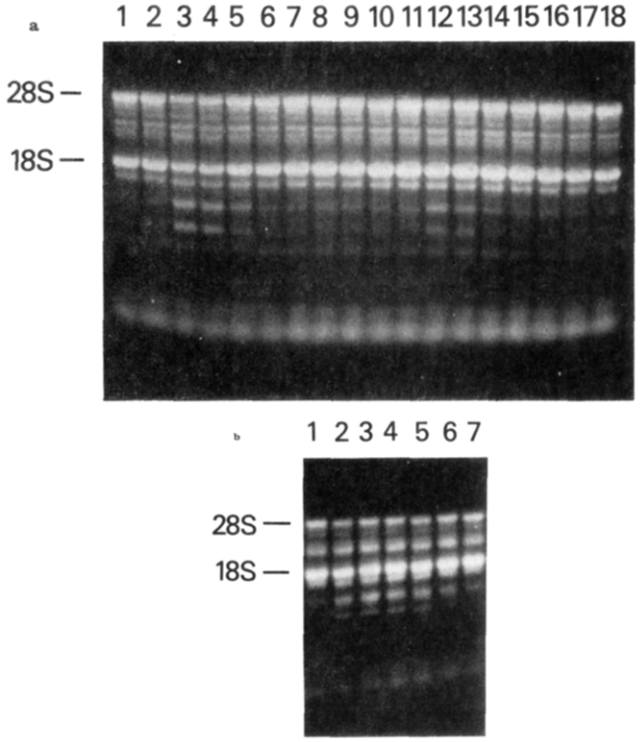

FIGURE 4: Ribosomal RNA cleavage assays for RNase L activity of 2–5A and various tubercidin analogues. (a) Tracks 1 and 7, controls, no additions; tracks 3–6, 2×10^{-7}, 2×10^{-8}, 2×10^{-9} and 2×10^{-10} M ppp5′A2′p5′A2′p5′A, respectively; tracks 8–11, 2×10^{-6}, 2×10^{-7}, 2×10^{-9} M ppp5′A2′p5′(c^7A)2′p5′(c^7A), respectively; tracks 12–15, 2×10^{-4}, 2×10^{-6}, 2×10^{-8}, and 2×10^{-10} M ppp5′(c^7A)2′p5′A2′p5′A; respectively; tracks 16–18, 2×10^{-6}, 2×10^{-8}, and 2×10^{-10} ppp5′A2′p5′A2′p5′(c^7A), respectively. (b) Tracks 1 and 8, controls, no additions; tracks 2–7, 2×10^{-5}, 2×10^{-6}, 2×10^{-7}, 2×10^{-8}, 2×10^{-9}, and 2×10^{-10} M ppp5′A2′p5′(c^7A)2′p5′A, respectively.

centrations remaining were determined by HPLC at 0, 20, 40, and 90 min. We could not detect any significant change in the concentration of any of the analogues nor of 2–5A itself over the 90-min incubation period (data not illustrated).

Discussion

It should be borne in mind that the following discussion will apply only to the RNase L derived from mouse L cells since there are some significant differences in behavior of the all-tubercidin-substituted derivatives, ppp5′(c^7A)2′p5′(c^7A)-2′p5′(c^7A) or corresponding tetramer, when the source of RNase L is rabbit reticulocytes or human cells. Thus, ppp5′(c^7A)2′p5′(c^7A)2′p5′(c^7A)2′p5′(C^7A) is just as effective an inhibitor of translation as 2–5A tetramer in rabbit reticulocyte lysates (Jamoulle et at., 1984), and ppp5′(c^7A)2′p5′-(c^7A)2′p5′(c^7A) can effect rRNA cleavage in extracts of human Daudi lymphoblastoid cells or human HeLa cells albeit 10–100 times less effectively than 2–5A (Lesiak, Jamoulle, and Torrence, unpublished observations).

Examination of the RNase L binding results (Figure 2 and Table IV) revealed relatively small differences. A trend seemed to exist that substitution of 7-deazaadenosine for adenosine on the 2′-terminal or third residue of 2–5A gave the greatest drop in binding ability [i.e., ppp5′(c^7A)2′p5′(c^7A)-2′p5′(c^7A), ppp5′A2′p5′(c^7A)2′p5′(c^7A), and ppp5′A2′p5′A2′p5′(c^7A)]. Replacement of the 5′-terminal or the second adenosine unit with the tubercidin moiety had much less effect, if any. It does not seem likely that the purine N7 moiety of either the first or second nucleotide residues of 2–5A plays an important role in endonuclease binding. There may exist some binding contributions of the purine N7 of the third adenosine nucleitide residue, but it is not nearly as critical a contribution as, for instance, the adenosine N1–N6 functionality of the 5′-terminal residue (Imai et al., 1985).

Table IV: Summary of Radiobinding and Protein Synthesis Inhibition Data[a]

analogue	radiobinding, IC_{50} (M)	protein synthesis, $IC_{1/2,max}$ (M)
ppp5′A2′p5′A2′p5′A	3×10^{-10}	6×10^{-10}
ppp5′(c^7A)2′p5′A2′p5′A	1×10^{-9}	4×10^{-9}
ppp5′A2′p5′(c^7A)2′p5′A	5×10^{-10}	5×10^{-10}
ppp5′A2′p5′A2′p5′(c^7A)	6×10^{-9}	2×10^{-8}
ppp5′A2′p5′(c^7A)2′p5′(c^7A)	5×10^{-9}	2×10^{-8}
ppp5′(c^7A)2′p5′(c^7A)2′p5′(c^7A)	5×10^{-9}	$>10^{-5}$

[a] See text for further details.

The purine N7 moieties play a central role in activation of the mouse L cell enzyme as documented earlier (Jamoulle et al., 1984). Whenever the first and/or third adenosine residue of 2–5A was replaced by tubercidin, there was a dramatic decrease in ability to activate RNase L as judged by either inhibition of translation or rRNA cleavege (Figures 3 and 4 and Table IV). Replacement of the purine ring of third (2′-terminal) nucleotide by the 7-deazapurine system was most effective at abolishing activity [i.e., ppp5′A2′p5′A2′p5′(c^7A), ppp5′A2′p5′(c^7A)2′p5′(c^7A), and ppp5′(c^7A)2′p5′(c^7A)-2′p5′(c^7A)]. The analogue in which the 5′-terminal adenosine was replaced by tubercidin, ppp5′(c^7A)2′p5′A2′p5′A, retained a bit more activity but was nonetheless about 100-fold less active than 2–5A. Only one analogue, ppp5′A2′p5′(c^7A)-2′p5′A, in which the second adenosine nucleotide unit was replaced by a tubercidin nucleotide, retained activity equivalent to 2–5A itself.

Parenthetically it may be noted that the inability of certain 2–5A analogues to inhibit translation to the same extent as 2–5A regardless of the concentration employed [e.g., ppp5′-(c^7A)2′p5′A2′p5′A, Figure 3) has been observed previously (Eppstein et al., 1985; Lesiak & Torrence, 1986; Lesiak and Torrence, unpublished observations). In previous instances, this anomalous behavior appeared to be related to the kinetics of inhibition of protein synthesis by such analogues.

The simplest interpretation of the above results (see Table IV) is that the purine N7 moieties of the first (5′-terminal) and third (2′-terminal) adenosine nucleotide residues of 2–5A are involved in activation of RNase L. This result is, in some respects, similar to the case of the inosine-substituted sequence isomers of 2–5A (Imai et al., 1985): the second nucleotide unit of 2–5A seems to play at best a minor role in endonuclease binding and activation whereas the two termini of the molecule are the "business" ends. One possibility to be considered would be that, in concert with the results of Imai et al. (1985), a critical binding interaction takes place between some domain of RNase L and N1 and/or the exocyclic amino group of the 5′-terminal residue of 2–5A. In order for activation to occur, however, some domain of RNase L must interact with the purine N7 group of the same (5′-terminal) adenosine residue. This kind of interaction conceivably could be mediated through asparagine or glutamine residues in the activator site of RNase L. On the other hand, also in agreement with the data of Imai et al. (1985), neither the adenosine N1 and/or the exocyclic 6-amino group nor the adenosine N7 moiety of the third or 2′-terminal nucleotide of 2–5A appears to be involved in binding of 2–5A to RNase L; however, both such residues must interact with some region in RNase L in order for nuclease activation to occur. Again, possible candidates in the activation binding site of RNase L may be asparagine or glutmaine residues.

Viewed from the perspective of the adenosine N1 and/or 6-amino group and the N7 position, the second adenosine moiety of 2–5A seems not to be of vital importance. Might it simply be serving the role of a spacer between the first and third residues that seem to be chiefly involved in binding and/or activation? To test this possibility, further studies are in progress in this laboratory to ascertain the role of the remainder of the adenine ring as well as the ribose residue of the second adenosine nucleotide of the 2–5A molecule.

Registry No. p5′A2′p5′(c^7A)2′p5′(c^7A), 105882-97-1; p5′A2′p5′A2′p5′(c^7A), 105882-98-2; p5′(c^7A)2′p5′A2′p5′A, 105882-99-3; p5′A2′p5′(c^7A)2′p5′A, 105883-00-9; A3′p5′A2′p5′-(c^7A)2′p5′(c^7A), 105883-01-0; A3′p5′A, 102029-96-9; Imp5′(c^7A)-2′p5′(c^7A), 105900-48-9; A3′p5′A3′p5′(c^7A)2′p5′(c^7A), 105883-02-1; A2′p5′A·NH_3, 102185-12-6; Imp5′(c^7A), 105883-03-2; A2′p5′A2′p5′(c^7A), 90041-96-6; Mop5′(c^7A), 89681-87-8; Imp5′A2′p5′A, 95314-01-5; Mop5′(c^7A)2′p5′A2′p5′A, 105900-49-0; Mop5′(c^7A)3′p5′A2′p5′A, 105883-04-3; Mop5′A, 20816-58-4; Mop5′(c^7A)2′p5′A, 105883-05-4; p5′(c^7A)2′p5′A, 105883-06-5; Imp5′(c^7A)2′p5′A, 105883-07-6; Mop5′A2′p5′(c^7A)2′p5′A, 105883-08-7; Imp5′A2′p5′(c^7A)2′p5′(c^7A), 105883-12-3; ppp5′A2′p5′(c^7A)2′p5′(c^7A), 102254-94-4; ppp5′A2′p5′A2′p5′A, 65954-93-0; ppp5′(c^7A)2′p5′A2′p5′A, 102254-91-1; ppp5′A2′p5′-(c^7A)2′p5′A, 102254-92-2; ppp5′A2′p5′A2′p5′(c^7A), 102254-93-3; ppp5′(c^7A)2′p5′(c^7A)2′p5′(c^7A), 84824-03-3; A3′p5′A2′p5′(c^7A), 105883-09-8; p5′A2′p5′(c^7A), 105883-10-1; Imp5′A2′p5′(c^7A), 105900-50-3; A3′p5′A2′p5′A2′p5′(c^7A), 105883-11-2; RNase L, 76774-39-5.

References

Eppstein, D. A., Van Der Pas, M. A., Schryver, B. B., Sawai, H., Lesiak, K., Imai, J., & Torrence, P. F. (1985) *J. Biol. Chem. 260*, 3666–3671.

Golgher, R. R., Williams, B. R. G., Gilbert, C. S., Brown, R. E., & Kerr, I. M. (1980) *Ann. N.Y. Acad. Sci. 350*, 448–458.

Imai, J., & Torrence, P. F. (1985) *J. Org. Chem. 50*, 1418–1420.

Imai, J., Lesiak, K., & Torrence, P. F. (1985) *J. Biol. Chem. 260*, 1390–1393.

Jamoulle, J. C., Imai, J., Lesiak, K., & Torrence, P. F. (1984) *Biochemistry 23*, 3063–3069.

Johnston, M. I., & Torrence, P. F. (1984) in *Interferon: Mechanisms of Production and Action* (Friedman, R. M., Ed.) pp 189–298, Elsevier, Amsterdam.

Kerr, J. M., & Brown, R. E. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 256–260.

Knight, M., Cayley, P. J., Silverman, R. H., Wreschner, D. H., Gilbert, C. S., Brown, R. E., & Kerr, I. M. (1980) *Nature (London) 288*, 189–192.

Krause, D., Silverman, R. H., Jacobsen, H., Leisy, S., Dieffenbach, C., & Friedman, R. M. (1985) *Eur. J. Biochem. 146*, 611–618.

Lesiak, K., & Torrence, P. F. (1986) *J. Med. Chem. 29*, 1015–1022.

Lesiak, K., Imai, J., Floyd-Smith, G., & Torrence, P. F. (1983) *J. Biol. Chem. 258*, 13082–13088.

Nilsen, T. W., Maroney, P. A., Robertson, H. D., & Baglioni, C. (1982) *Mol. Cell. Biol. 2*, 154–160.

Sawai, H., & Ohno, M. (1981a) *Bull. Chem. Soc. Jpn. 54*, 2752–2754.

Sawai, H., & Ohno, M. (1981b) *Chem. Pharm. Bull. 29*, 2237–2245.

Sawai, H., Shibata, T., & Friedman, R. M. (1979) *J. Biol. Chem. 254*, 1259–1267.

Torrence, P. F., Imai, J., Lesiak, K., Jamoulle, J.-C., & Sawai, H. (1984a) *J. Med. Chem. 27*, 726–733.

Torrence, P. F., Imai, J., Lesiak, K., Jamoulle, J.-C., Sawai, H., Warinner, J., Balzarini, J., & De Clercq, E. (1984b) in *Targets For The Design of Antiviral Agents* (De Clercq, E., & Walker, R. T., Eds.) pp 259–285, Plenum, New York.

Torrence, P. F., Imai, J., Jamoulle, J.-C., & Lesiak, K. (1986a) *Chem. Scr. 26*, 191–197.

Torrence, P. F., Imai, J., Jamoulle, J.-C., & Lesiak, K. (1986b) *Methods Enzymol. 119*, 522–529.

Williams, B. R. G., Golgher, R. R., Brown, R. E., Gilbert, C. S., & Kerr, I. M. (1979) *Nature (London) 282*, 582–585.

Wreschner, D. H., James, T. C., Silverman, R. H., & Kerr, I. M. (1981) *Nucleic Acids Res. 9*, 1571–1581.