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An interferon-induced phosphodiesterase degrading (2′-5′)oligoisoadenylate and the C-C-A terminus of tRNA

(interferon action/translation control/tRNA aminoacylation/2′-5′-nucleotide bonds)

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ABSTRACT **A phosphodiesterase characterized by a generally higher activity on 2′-5′ than on 3′-5′ phosphodiester bonds was isolated from mouse L cells treated with interferon. A similar enzyme was purified from mouse reticulocytes. The phosphodiesterase 2′-PDi splits the 2′-phosphate bond of pppA2′p5′A2′p5′A, the oligonucleotide activator of ribonuclease F. The level of phosphodiesterase 2′-PDi is increased by interferon treatment of L cells. The phosphodiesterase was also shown to degrade the C-C-A terminus of tRNA and to reduce the amino acid acceptance of tRNA in cell-free extracts, thereby causing a tRNA-reversible inhibition of mRNA translation.**

In extracts of interferon-treated cells, translation of mRNA into proteins is inhibited by several enzymatic pathways (1–3). One pathway is mediated by the double-stranded RNA (dsRNA)/ATP-activated protein kinase PK-i, induced by interferon in the cells (1, 3), which phosphorylates eukaryotic initiation factor 2 and decreases Met-tRNA$_f^{Met}$ binding to 40S ribosomes (4). A second translational-inhibitory pathway is mediated through synthesis of (2′-5′)pppApApA (5) by the dsRNA/ATP-dependent oligoisoadenylate synthetase E, another interferon-induced enzyme (1, 3, 6, 7). The oligonucleotide enhances mRNA degradation in cell extracts (8, 9), and its synthesis gives rise to the apparent dsRNA/ATP-dependent endonucleolytic activity of interferon-treated cell extracts (3, 10–12). This function of the oligonucleotide was demonstrated by the isolation of RNase F, whose nucleolytic activity is completely dependent on the continued presence of (2′-5′)pppApApA, from both interferon-treated and untreated cells (13). The third pathway of translation inhibition does not require dsRNA, affects polypeptide chain elongation, and is reversed by the addition of tRNA (14–20). The mechanism that brings about this tRNA deficiency in interferon-treated cell extracts has not been elucidated.

In the study of the oligoisoadenylate-dependent RNase F, we found (13) a phosphodiesterase that cleaves the (2′-5′)-pppApApA oligonucleotide into ATP and 5′-AMP, and inactivates RNase F. Degradation of (2′-5′)pppApApA in crude cell extracts was also observed by others (21, 22). We report here the purification of this phosphodiesterase from interferon-treated L cells and mouse reticulocytes. The enzyme is a 40,000 M_r protein with a generally higher affinity for 2′-5′ dinucleotides than for their 3′-5′ isomers. The phosphodiesterase, however, also degrades the C-C-A terminus of tRNA, thereby causing a tRNA-reversible inhibition of protein synthesis in the cell-free systems. We find a 4- to 5-fold increase in this 2′-phosphodiesterase activity in L cells after interferon treatment. This increased activity could explain the tRNA deficiency and elongation block observed in extracts of interferon-treated cells. In view of its unusual substrate specificity and enhancement by interferon, we call this phosphodiesterase 2′-PDi.

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MATERIALS AND METHODS

Assay and Purification of 2′-PDi. Assays are described in the legend of Fig. 1. Routinely, degradation of (2′-5′)ApA was measured in 15 mM Hepes buffer, pH 7.5/90 mM KCl/3.5 mM $MgCl_2$/0.7 mM dithiothreitol/7% (vol/vol) glycerol. After chromatography on polyethyleneimine (PEI)-cellulose, the nucleotides were eluted with 0.7 M $MgCl_2$/20 mM Tris·HCl, pH 7.5, and absorbance at 260 nm was measured. Purification of 2′-PDi from mouse L cells treated 24 hr with 200 units of interferon per ml (1) is outlined in Table 1. Steps I–III were as for RNase F (13). At step IV, 2′-PDi eluted from hydroxylapatite at 50 mM K phosphate, whereas RNase F eluted at higher phosphate (13). For mouse reticulocytes (23), the 100,000 × *g* supernatant (100 ml) was loaded on 300 ml of DEAE-cellulose in 20 mM Hepes buffer, pH 7.5/25 mM KCl/5 mM $MgCl_2$/1 mM dithiothreitol/10% (vol/vol) glycerol (buffer A). Proteins eluted by 150 mM KCl in buffer A (step IIa, 300 mg, 50 ml) were applied to a DEAE-cellulose column (1.6 × 15 cm) and eluted with a 200-ml 25–150 mM KCl gradient in buffer A. Fractions eluting at 60–80 mM KCl (step IIb, 150 mg, 60 ml) were loaded on phosphocellulose (1.2 × 14 cm) in buffer A but at pH 6.7 and with no Mg^{2+}, and were eluted with a 160-ml 25–800 mM KCl gradient. Fractions around 280 mM KCl (step III, 60 mg, 40 ml) were applied to hydroxylapatite (1.6 × 10 cm) in 25 mM K phosphate buffer, pH 7.2/120 mM KCl/1 mM dithiothreitol. The 2′-PDi was eluted stepwise with 100 mM phosphate (step IV), while RNase F eluted in the 150 mM phosphate step. The 2′-PDi (step IV, 2.5 mg, 13 ml) was applied to a blue dextran-Sepharose (24) column (1 × 3.2 cm) and eluted by an 18-ml 120–700 mM KCl gradient in buffer A. Activity eluted around 200 mM KCl (step V, 0.4 mg) and 0.24 mg in 0.4 ml was filtered through Bio-Gel P-100 (0.7 × 60 cm) in buffer A with 120 mM KCl (step VI, Fig. 2*A*).

Effect of 2′-PDi on tRNA Activity. Aminoacyl-tRNA synthetases were prepared from rabbit reticulocytes by pH 5 precipitation and DEAE-cellulose chromatography (16, 25). Rat liver tRNA nucleotidyltransferase was partially purified as in ref. 26. Rabbit liver tRNA, 1.2 μg, was preincubated 10 min at 30°C, in 10 μl of 15 mM Hepes buffer, pH 7.5/3.5 mM $MgCl_2$/90 mM KCl/0.7 mM dithiothreitol/7% (vol/vol) glycerol with the indicated amounts of 2′-PDi. After heating 10 min at 60°C, the reaction mixture was adjusted to 50 μl of 30 mM Hepes buffer, pH 7.5/1 mM $MgCl_2$/80 mM KCl/2 mM dithiothreitol/0.01 mM EDTA/3% (vol/vol) glycerol/1 mM ATP/0.25 mM GTP/0.25 mM CTP (when indicated)/5 mM creatine phosphate/80 μg of creatine kinase per ml/60 μM each of 19 amino acids/4 μM [^{3}H]leucine (50 Ci/mmol; 1 Ci = 3.7 × 10^{10} becquerels)/90 μg of aminoacyl-tRNA synthetase/0.01 unit of tRNA nucleotidyltransferase (when indicated). Incu-

Abbreviations: dsRNA, double-stranded RNA; 2′-PDi, interferon-induced 2′-phosphodiesterase; PEI-cellulose, polyethyleneimine-cellulose.

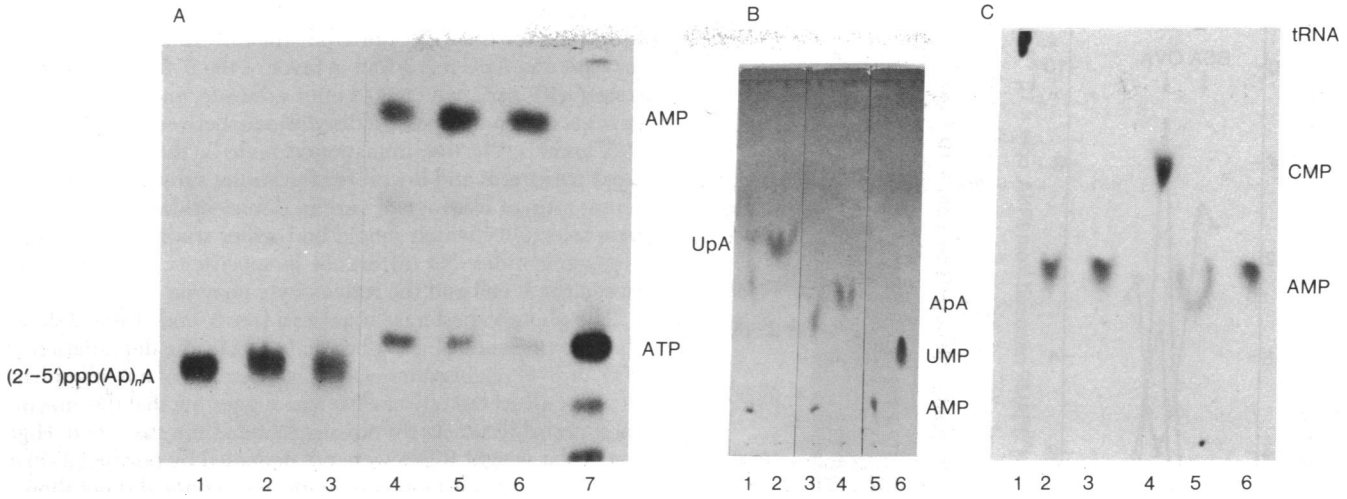

FIG. 1. Phosphodiesterase activity of 2′-PDi. (*A*) Cleavage of oligoisoadenylate: Enzymatically synthesized (2′-5′)ppp(Ap)$_n$A oligomers, labeled by ^{32}P in the α-phosphate, were prepared as described (13). Twenty picomoles of the dimer and trimer and 10 pmol of tetramer (between 1000 and 2000 cpm) were incubated as outlined in *Materials and Methods* in 10 μl for 30 min at 30°C with 0.4 μg of 2′-PDi from interferon-treated cells (step III), and chromatographed on PEI-cellulose with a 0.75 M potassium phosphate buffer, pH 3.5. The autoradiogram is shown. Lanes 1–3 are di-, tri-, and tetramer, respectively, without 2′-PDi; lanes 4–6 are di-, tri-, and tetramer digested with 2′-PDi; lane 7 is a radioactive ATP marker. The positions of unlabeled AMP and (2′-5′)ppp(Ap)$_n$A markers are indicated.

(*B*) Cleavage of 2′-5′ dinucleoside monophosphates: Chemically synthesized (2′-5′)UpA, 4.6 nmol, and ApA, 3.3 nmol, were incubated in 8 μl for 60 min at 37°C with 2 μg of 2′-PDi from reticulocytes (step III) and chromatographed on PEI-cellulose with 0.32 M LiCl. The UV photograph shows in lanes 1 and 3 (2′-5′)UpA and ApA digested by 2′-PDi; in lanes 2 and 4, the same without 2′-PDi; lanes 5 and 6, AMP and UMP markers.

(*C*) Cleavage of tRNA-CpC[^{32}P]pA: tRNA terminally labeled with [α-^{32}P]ATP (4 μg, 820 cpm) was incubated in 15 μl for 60 min at 37°C alone (lane 1), with 0.5 μg of reticulocyte 2′-PDi from step V (lanes 2 and 3), or with 1.5 μg of pancreatic RNase (lane 4), 15 μg of snake venom phosphodiesterase (lane 5), or 0.9 μg of 2′-PDi from interferon-treated cells (step III)(lane 6) before chromatography on PEI-cellulose in 1.8 M LiCl, pH 7.9, and autoradiography. Positions of nonradioactive markers are shown.

bation was continued 30 min at 30°C and radioactive material insoluble in cold trichloroacetic acid was determined. To prepare tRNA-CpC[^{32}P]pA, 20 μg of tRNA was preincubated as above with 1.4 μg of 2′-PDi (step IV) for 45 min, and in the second incubation 10 μCi of [α-^{32}P]ATP (1 Ci/mmol) was added while leucine and synthetase were omitted. The tRNA was extracted with phenol, precipitated with ethanol, and filtered through a Sephadex G-50 syringe.

RESULTS

Isolation of 2′-PDi. The 2′-5′ phosphodiester bonds of oligoisoadenylate are resistant to common ribonucleases (5), but we found (13) that L cells contain an enzyme that degrades (2′-5′)pppApApA into ATP and 5′-AMP (Fig. 1*A*). This enzyme also splits the 2′-phosphate bond of synthetic ApA (Fig. 1*B*), and we used the degradation of this substrate as the assay for purification. One unit of this phosphodiesterase is defined as the cleavage of 1 nmol of (2′-5′)ApA into 5′-AMP, in 60 min at 37°C.

Table 1. Purification of 2′-PDi from interferon-treated mouse L cells and from mouse reticulocytes

Step	2′-PDi specific activity, units/mg protein: Interferon-treated L cells	2′-PDi specific activity, units/mg protein: Mouse reticulocytes
I. 100,000 × *g* supernatant	95	—*
IIa. DEAE-cellulose, step elution	—†	150
IIb. DEAE-cellulose, gradient elution	1,670	320
III. Phosphocellulose	9,600	1,000
IV. Hydroxylapatite	34,200	6,000
V. Blue dextran-Sepharose	—†	14,000
VI. Bio-Gel P-100	—†	21,300

*Not accurately measured.
†Step omitted.

The enzyme was purified from extracts of L cells treated with interferon, and also from mouse reticulocytes. The data summarized in Table 1 show that the phosphodiesterase that splits the 2′-phosphate bond of (2′-5′)ApA can be purified about 300-fold from cell sap of interferon-treated L cells. In reticulocytes, the purification factor was similar but the activity could not be accurately measured in step I because of hemoglobin; recovery from step II to step V was 12%. The elution pattern of reticulocyte enzyme for Bio-Gel P-100 (step VI) is shown in Fig. 2*A*. As compared to known molecular weight markers, the enzyme elutes as a 40,000 M_r protein. A similar size was determined for the native enzyme upon centrifugation on a 5–20% glycerol gradient (not shown). Electrophoresis of the active fraction on a polyacrylamide gel in dodecylsulfate (Fig. 2*B*) shows a main band at 35,000 M_r. A higher molecular weight contaminant is still present but does not correspond to the 2′-phosphodiesterase activity (Fig. 2*B*). The L cell enzyme was obtained at step IV in the same purity as the reticulocyte enzyme, and showed a single 35,000 M_r band by dodecyl sulfate gel electrophoresis.

Substrate Specificity of 2′-PDi. The phosphodiesterase purified by measuring the cleavage of (2′-5′)ApA degrades the natural (2′-5′)pppApApA oligoisoadenylate trimer (Fig. 1*A*). The products of degradation are 5′-AMP and ATP; no 2′- or 3′-AMP was detected by electrophoresis at pH 3.5 (13), and only very small amounts of ADP were seen. Isoadenylate dimers yielded 1 ATP per AMP, while the expected higher ratios of AMP to ATP were obtained from trimers and tetramers as shown in Fig. 1*A*. 2′-PDi resembles snake venom phosphodiesterase in its ability to cleave the 2′-phosphate bond, but differs from it because it does not hydrolyze ATP, does not cleave deoxyribonucleotides (Table 2), and exhibits a greater substrate specificity. The activity of 2′-PDi was measured on a series of

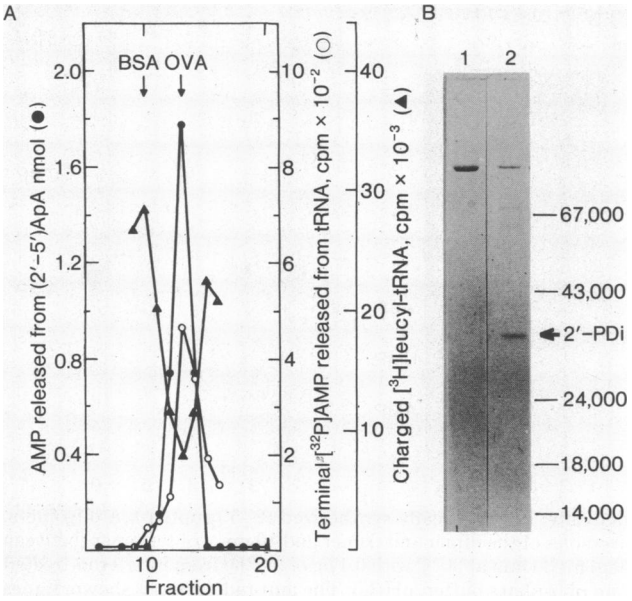

FIG. 2. Purification of 2'-PDi. (A) Filtration on Bio-Gel P-100. Step VI in the purification of the reticulocyte enzyme is shown. Cleavage of (2'-5')ApA into AMP was measured with 10 μl of each fraction (●). Inhibition of tRNA aminoacylation was measured after preincubation of rabbit liver tRNA with 5 μl of each fraction (▲). Release of [^{32}P]AMP from the C-C-A terminus of tRNA was measured as in Fig. 1*C* (○). Position of known molecular weight protein markers bovine serum albumin (BSA) and ovalbumin (OVA) is shown. (B) Polyacrylamide gel electrophoresis in sodium dodecyl sulfate. Staining was with Coomassie blue. The peak fraction of 2'-PDi activity (fraction 12) from a glycerol gradient centrifugation (of 20 fractions) is shown in lane 2. The upper band is clearly a contaminant because it is found in a heavier fraction devoid of phosphodiesterase activity (fraction 8, lane 1). The right-hand scale shows molecular weight markers.

2'-5' dinucleoside monophosphates and their 3'-5' isomers (generous gifts from S. Rapoport and Y. Lapidot, Jerusalem). Rate measurements were done under limiting amount of purified enzyme, and activity was calculated as the release of nucleoside 5'-monophosphate (Fig. 1*B*). Table 2 shows that in general the enzyme prefers the 2'-5' isomer. Thus, for CpA and UpU the rate of cleavage is about 30 times faster for the 2'-5' form than for the 3'-5'. For UpA, the difference is 6-fold and for ApA and ApU it is 3-fold in favor of the 2'-5'. An exception is seen with ApC, which is a better substrate, and for which the enzyme does not appear to discriminate between the 2'-5' and 3'-5' isomers. The discrimination seems to be the highest for the weak substrates and lowest for the strong substrates. The different rates of cleavage of various dinucleotides suggests some base selectivity which should be further studied with longer oligonucleotides. No difference in specificity was found between the L cell and the reticulocyte enzyme.

Table 2. Substrate specificity of 2-'PDi

Substrate	Nucleotide produced	Efficiency of cleavage, nmol product/nmol substrate	
		2'-5' isomer	3'-5' isomer
ApA	5'-AMP	0.41	0.12
UpA	5'-AMP	0.44	0.07
CpA	5'-AMP	0.19	0.005
ApU	5'-UMP	0.41	0.13
UpU	5'-UMP	0.33	0.01
ApC	5'-CMP	0.74	0.65
ApG	5'-GMP	ND	0.05
pApA	5'-AMP	0.73*	ND
pApApApA	5'-AMP	0.63*	ND
ApAp	(pAp)	0	ND
ApApApAp	(pAp or 5'-AMP)	0	0
dApdA	(dAMP)	—	0

Reactions proceeded for 60 min at 37°C with 0.4 μg of 2'-PDi (step V). The reticulocyte and interferon-treated cell enzymes gave identical results. About 10 nmol of substrate was used per assay. The yield of each nucleotide was calculated from the known extinction coefficient at 260 nm. Parentheses indicate expected products; ND, not done.
* Two and 4 nmol of AMP are expected per nmol of pApA and pApApApA, respectively.

The phosphodiesterase requires a free 3' end: Table 2 shows that the presence of a 3' phosphate blocks the degradation of 2'-5' or 3'-5' oligoisoadenylate. The presence of a 5' phosphate does not affect the rate of cleavage, suggesting that the enzyme most probably attacks the oligonucleotide from the 3' end. High molecular weight RNAs were not degraded by purified 2'-PDi: Mengo virus RNA incubated with the enzyme did not show a significantly altered electrophoretic mobility. Less purified preparations of 2'-PDi were, however, contaminated by an endonuclease, but this RNase did not cleave (2'-5')ApA, did not inactivate tRNA, and was clearly separated from 2'-PDi. It is possible that 2'-PDi degrades the 3' end of mRNA, because some AMP was released from [^{32}P]poly(A)$^+$ mRNA. Furthermore, we have obtained evidence that the enzyme inactivates tRNA by removing its 3' terminus.

Effect of 2'-PDi on tRNA Activity. Rabbit liver tRNA was labeled at the 3'-terminal A residue by using tRNA nucleotidyltransferase and [α-^{32}P]ATP. When such tRNA-CpC[^{32}P]pA was incubated with the most purified preparations of 2'-PDi, all the label was released as 5'-AMP (Fig. 1*C*). The electrophoretic mobility of the tRNA was unchanged by this treatment (not shown). In comparison, pancreatic RNase A released all the ^{32}P as CMP (Fig. 1*C*), whereas snake venom phosphodiesterase released all the label as 5'-AMP, confirming that the ^{32}P was indeed in the last phosphodiester bond of the C-C-A terminus of tRNA.

Because (3'-5')CpA is a rather poor substrate for the 2'-PDi enzyme (Table 2), it was important to ascertain that both release of the terminal AMP of tRNA and cleavage of oligoisoadenylate are due to the same enzyme. Both activities copurified through the various steps of Table 1, and Fig. 2 shows that both activities elute identically from the Bio-Gel column used as the last step of purification. Both activities were maximum at pH 7.5–8, 80 mM KCl, and 3–9 mM $MgCl_2$. Furthermore, heat inactivation experiments showed that at 55°C both activities were lost in 10 min with the same kinetics (not shown). Substrate competition experiments also supported the identity between the two activities: Addition of 8 nmol of (2'-5')UpU to a reaction mixture containing 0.3 nmol of tRNA inhibited by 90% the release of 5'-AMP from the tRNA; in contrast, (3'-5')UpU gave less than 30% inhibition. Finally, both activities of the 2'-PDi required free 2'-(3'-)OH groups on the substrate: [^{3}H]leucyl-tRNA was not hydrolyzed by 2'-PDi. Another protein, deacylating tRNA, was separated from 2'-PDi at step IV of purification. Oligoisoadenylate-dependent RNase F did not degrade or inactivate tRNA (not shown).

2'-PDi degrades only the C-C-A terminus of tRNA. Preincubation of tRNA with the enzyme for 5–10 min inhibited subsequent aminoacylation with leucine (Table 3), but the readdition of C-C-A by tRNA nucleotidyltransferase fully restored charging. For these experiments, the 2'-PDi was heat inactivated after incubation with tRNA and prior to the addition of the aminoacyl-tRNA synthetase. We verified that the nucleotidyltransferase used was free from tRNA or from aminoacyl-tRNA synthetase. Addition of CTP further helped to

Table 3. Effect of 2′-PDi on synthesis of Leu-tRNA

Addition to preincubation		Addition to incubation			Leu-tRNA synthesis, pmol	
tRNA	2′-PDi	Aminoacyl-tRNA synthetase	Nucleotidyl-transferase	CTP	Exp. 1	Exp. 2
+	−	+	−	−	6.2	4.7
+	−	−	+	−	0.3	0.3
+	−	+	+	−	7.2	4.9
+	−	+	+	+	7.2	ND
+	+	+	−	−	1.0	0.3
+	+	−	+	−	0.2	0.07
+	+	+	+	−	5.3	3.5
+	+	+	+	+	7.0	ND

ATP was present in all cases. Exp. 1: 2′-PDi from mouse reticulocyte, 2 μg of step IV. Exp. 2: 2′-PDi from interferon-treated cells, 1.8 μg of step III. A total of 50 pmol of tRNA was used. Background values without tRNA were subtracted. ND, not done.

restore the activity of the 2′-PDi-treated tRNA by nucleotidyltransferase (Table 3). We calculated that, when about 80% of the tRNA is degraded by 2′-PDi, about 30% is degraded beyond the terminal A and requires CTP for repair; only 4% of the tRNA could not be repaired by the tRNA nucleotidyltransferase. Incubation of synthetic (3′-5′)ApCpC with 2′-PDi also released 5′-CMP at the same rate as (3′-5′)ApA or oligo(A) was cleaved. The effect of 2′-PDi on tRNA does not appear to be specific for a given amino acid, and the inhibition of acylation by a mixture of 16 amino acids was similar to that by leucine (not shown). During purification, the inhibition of tRNA aminoacylation and the (2′-5′)ApA degradation activity coincided (Fig. 2*A*). Furthermore, addition of 1 mM (2′-5′)ApA could reduce by 50% the inactivation of tRNA by 2′-PDi.

Effect of 2′-PDi on Protein Synthesis. L cell S10 extracts (preincubated and Sephadex-filtered 10,000 × *g* supernatants as in ref. 14) showed a decreased aminoacylation of their endogenous tRNAs after incubation in the presence of 2′-PDi, as demonstrated in Table 4 for leucine tRNA. This was accompanied by a decreased ability of the S10 extracts to translate Mengo virus RNA (Table 4), which was confirmed by analysis of the translation products by gel electrophoresis (not shown). Addition of exogenous rabbit liver tRNA was able to restore Mengo virus RNA translation in S10 extracts incubated with the phosphodiesterase 2′-PDi. This enzyme, can, therefore, produce in extracts from untreated L cells a tRNA-reversible translation inhibition similar to that seen in extracts of interferon-treated cells in the absence of dsRNA (16, 20).

Increase in 2′-PDi after Treatment of L Cells with Interferon. We used the degradation of chemically synthesized (2′-5′)ApA into 5′-AMP to assay (under conditions of excess substrate) the level of 2′-PDi in 10,000 × *g* supernatants prepared from L cell monolayers. Calculated in units of 2′-PDi per mg of protein, the activity of the 2′-phosphodiesterase was 75 in extracts of untreated cells. When measured 8 hr after the addition to the L cells of mouse interferon at 5 units/ml, the activity of 2′-PDi was 240 units/mg of protein; with interferon on the cell culture at 125 units/ml, the phosphodiesterase activity was 325 units/mg of protein. Vesicular stomatitis viral RNA synthesis measured (by the method described in ref. 27), after infection of duplicate cultures of L cells, was reduced by 88% with interferon at 5 units/ml and by 98% with interferon at 125 units/ml. A kinetic study of phosphodiesterase 2′-PDi increase after interferon treatment is reported elsewhere (28).

DISCUSSION

This paper describes the purification and characterization of an enzyme from mouse cells that cleaves the 2′-phosphate bond of interferon-induced (2′-5′)pppApApA. We find that this phosphodiesterase activity, measured in crude extracts with (2′-5′)ApA as substrate, increases when L cells have been pretreated by interferon. The failure of others (21, 22) to see an increase in oligoisoadenylate degradation may result from the difference in assay used. A similar enzyme is found in reticulocytes, which appear to contain many of the enzymes induced by interferon in other cells (29, 30). The enzyme is a phosphodiesterase that cleaves oligonucleotides from their 2′,3′ end and releases nucleoside 5′-monophosphates. The activity of the enzyme is generally higher on oligonucleotides with 2′-5′-phosphodiester bonds than on oligonucleotides with the 3′-5′ configuration, but this depends on the nature of the bases involved. Oligodeoxyribonucleotides are not cleaved. This 40,000 M_r enzyme, which we designate 2′-phosphodiesterase-interferon (2′-PDi), differs from snake venom phosphodiesterase (31) and from a phosphodiesterase observed in leukemia cells (32). The latter enzyme prefers substrates with a 5′ phosphate and degrades tRNA processively beyond its C-C-A end (33).

Activation of the cellular RNase F by interferon-induced (2′-5′)pppApApA is reversed by degradation of the oligonu-

Table 4. Effect of 2′-PDi on L cell extracts

Exp.	Preincubation Conditions	Preincubation Time, min	Leu-tRNA synthesis, [^{3}H]leucine cpm	Mengo virus RNA translation, [^{35}S]methionine cpm Without tRNA	With tRNA
1	S10	15	12,500	104,565	89,390
		60	9,885	—	—
	S10 + 2′-PDi	15	5,860	54,495	92,075
		60	2,835	—	—
2	S10	10	ND	140,575	232,940
	S10 + 2′-PDi	10	ND	89,830	224,565

Control L cell S10, 0.2 A_{260} unit, was preincubated in 20 μl with 35 mM Hepes buffer, pH 7.5/2.5 mM $MgCl_2$/120 mM KCl/2.5 mM dithiothreitol/5% glycerol/1.3 mM ATP/0.3 mM GTP/6 mM creatine phosphate/2 μg of creatine kinase/85 μM of each of 19 amino acids/(when indicated) 1.1 μg of 2′-PDi from interferon-treated cells (step III) (Exp. 1) or 2 μg of reticulocyte 2′-PDi (step IV) (Exp. 2) for the indicated time at 30°C. The volume was adjusted to 25 μl with 4 μM [^{3}H]leucine (50 Ci/mmol), and, after 30 min at 30°C, radioactivity insoluble in cold acid was measured in a 20-μl aliquot. For protein synthesis, 0.3 μg of Mengo virus RNA and 3 μM [^{35}S]methionine (600 Ci/mmol) were added and radioactivity insoluble in hot acid was measured after 60 min at 30°C in a 5-μl aliquot. Rabbit liver tRNA, 2 μg, was added where indicated. ND, not done.

cleotide by 2′-PDi (13). Activation of RNase F, therefore, lasts only as long as new (2′-5′)pppApApA is synthesized (13, 21, 22). The phosphodiesterase may serve to regulate the local concentration of (2′-5′)pppApApA and prevent a massive degradation of cellular RNA by RNase F. This fine regulation of RNase F by synthesis and degradation of its oligonucleotide activator may be very important for its role in the antiviral effect of interferon (34). Oligoisoadenylate dimers do not activate RNase F, but trimers, tetramers, and longer chains all have the same activity (5, 13). One could speculate that the chains of oligoisoadenylate are progressively cleaved from their 3′ end by 2′-PDi. When the chain becomes shorter than three A residues, RNase F activity stops. Such a hypothesis would provide a function for the longer oligoisoadenylate chains formed by the interferon-induced oligoisoadenylate synthetase (5, 13).

Purified 2′-PDi exhibits an additional effect on the protein synthesis system. The enzyme removes the C-C-A terminus of tRNA and produces upon preincubations of cell extracts a tRNA-reversible inhibition of mRNA translation. Because 2′-PDi increases after interferon treatment, we propose that this enzyme plays a role in the inhibition of polypeptide chain elongation observed in extracts of interferon-treated cells in the absence of added dsRNA (refs. 14–20; third pathway in the introduction). This elongation block was shown to be reversed by adding some minor species of tRNA (16). Although 2′-PDi is not selective for a specific tRNA, minor species present in low amounts in the cell would be more affected by a generalized decrease in tRNA activity. The tRNAs are not extensively degraded, because the activity of leucyl tRNA treated by 2′-PDi can be fully restored by the tRNA nucleotidyltransferase. In the cells, this repair function may regulate the activity of specific tRNAs. We have, indeed, observed a 2.5-fold higher turnover of the terminal A of tRNA in crude extracts of interferon-treated cells (unpublished), as expected from a higher degradation and repair of the C-C-A end of tRNA. The 2′-PDi does not degrade aminoacylated tRNA; it differs, therefore, from the deacylase reported by Sela *et al.* (35), but it is not excluded that several enzymes are involved in the tRNA deficiency.

In summary, the properties of 2′-PDi described here and its increase after interferon treatment may explain two features of the translation inhibition seen in extracts of interferon-treated cells. First, the enzyme may be needed to prevent excess accumulation of (2′-5′)pppApApA, the activator of RNase F in the cells. In addition, however, the phosphodiesterase would lower tRNA amino acid acceptance and be responsible for the translation inhibition seen in these extracts in the absence of dsRNA.

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