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Biochemistry

Kinetics of the induction of three translation-regulatory enzymes by interferon

(interferon action/translation control)

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ABSTRACT **Three enzymes that cause inhibition of mRNA translation, eukaryotic initiation factor 2 protein kinase PK-i, oligoisoadenylate synthetase E, and phosphodiesterase 2′-PDi, have been recently isolated from interferon-treated cells. We show that the rise in these three enzyme activities may be used to study the response of uninfected cells to interferon. For each enzyme, a specific microassay that can be carried out on extracts from 2–5 × 10^4 monolayer cells from mouse, monkey, or man was developed. With these assays, the kinetics of induction of the three enzymes in mouse L cells are compared. The dose dependence for protein kinase PK-i induction is shown to be similar to that for the development of the antiviral state. Actinomycin D and anti-interferon serum block enzyme induction if added to the cells early after interferon treatment. The quantitative measurements of the intracellular level of these enzymes provide a new and convenient model to study the cell's response to interferon.**

The biological activity of interferons has usually been studied by infecting treated cell cultures and measuring the inhibition of virus multiplication. A more direct approach would be to measure, in the cells, some interferon-induced biochemical changes associated with the establishment of the antiviral state. Several effects of interferon on cellular functions have been observed, such as reduction of cell proliferation or changes in cell membrane properties (1), but their molecular mechanisms have not been elucidated. One of the clearest biochemical alterations observed after interferon treatment is an impairment of viral protein synthesis (2–4). Moreover, several inhibitors of mRNA translation have been identified in interferon-treated cells and shown, after purification, to be enzymes that act on various components of the mRNA translation machinery. One enzyme is a specific protein kinase PK-i, phosphorylating a 67,000-M_r polypeptide (5–11) and the small subunit of eukaryotic initiation factor 2 (eIF-2) (12, 13). The purified protein kinase PK-i is activated by double-stranded RNA (dsRNA) and ATP, inhibits mRNA translation (13–15), and decreases met-tRNA binding to 40S ribosomes in L cell extracts (16). A phosphoprotein phosphatase that dephosphorylates the 67,000-M_r protein and eIF-2 was found as well (9, 15). A second inhibitor of translation is the oligonucleotide (2′-5′)pppApApA (17) formed by a dsRNA-dependent enzyme, the oligoisoadenylate synthetase E, in interferon-treated cells (6, 9, 13, 18, 19). The oligonucleotide enhances mRNA degradation (14, 20–22) by activating the (2′-5′)pppApApA-dependent ribonuclease F already present in untreated cells (23). An additional enzyme recently isolated (23) is a phosphodiesterase, 2′-PDi, which degrades 2′-5′ oligoisoadenylate to ATP and 5′-AMP. A purified preparation of this latter enzyme also degrades the -CpCpA terminus of tRNAs and inhibits protein synthesis (24).

In the present work, we have developed methods to assay specifically the level of these translation-regulatory enzymes in cytoplasmic extracts prepared from a minimal number of interferon-treated noninfected cells. With these assays, we show that it is possible to measure the increase of three enzymes within a few hours after interferon addition and study the kinetics of their induction. Measurement of the induction of these cellular enzymes provides a convenient model system to study the response of cells to interferon.

MATERIALS AND METHODS

Cell Cultures. Mouse L cells, lines CCL1 or L929, were grown as monolayers in GIBCO medium F16/8% calf serum/10% tryptose phosphate broth/100 units of penicillin per ml/0.1 mg of streptomycin per ml/0.4 mg of gentamycin per ml. We used either 60-mm plastic plates (2 × 10^6 cells in 4 ml) or 96-well microtiter plates (2–3 × 10^4 cells in 0.1 ml per well). One day after seeding, fresh medium with various doses of interferon was added. For kinetic experiments, 200 units of interferon per ml was added so that cells could be harvested simultaneously. Mouse interferon, produced in L cells by infection with Newcastle disease virus, was partially purified to 10^7 units/mg of protein by successive treatment with acid (pH 2), ammonium sulfate precipitation, and chromatography on CM-cellulose (5). Monkey and human interferon were produced and used on monkey BSC-1 and human FS11 cells as described (25).

Preparation of Cell Extracts. The plates were cooled on ice and washed three times with 35 mM Hepes, pH 7.5/140 mM NaCl/3 mM $MgCl_2$. To lyse the cells, 0.2 ml (for 60-mm plates) or 0.025 ml (for microplate wells) of 20 mM Hepes, pH 7.5/5 mM $MgCl_2$/120 mM KCl/1 mM dithiothreitol/10% glycerol (Buffer B) containing 0.5% Nonidet P40 (NP40) was added. After 6–9 min of agitation, the extracts from 60-mm plates were centrifuged for 6 min in an Eppendorf Microfuge (8000 × *g*). The supernatants (A_{280} about 17 units) could be stored in liquid air, and 2–5 μl was sufficient for measuring the various enzymatic activities. For microplates, the whole plate was centrifuged in an International swinging rotor at 3600 rpm for 15 min and 10–15 μl of the supernatant was used.

Assay of PK-i. Aliquots of cell extracts were preincubated in 20 μl of Buffer B/0.5 μg of poly(rI)·(rC) per ml/0.5 mM ATP for 15 min at 30°C. Then 5 μg of eIF-2 and 75 μM [γ-^{32}P]ATP (16 Ci/mmol; 1 Ci = 3.7 × 10^{10} becquerels) were added and the incubation (25 μl) was continued for 15 min at 30°C. Samples were submitted to electrophoresis in polyacrylamide gels in sodium dodecyl sulfate as before (5, 13). The autoradiographs were scanned at 600 nm in a Gilford recording spectrophotometer, and the area under the 35,000-M_r band of eIF-2 was measured in arbitrary units.

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Abbreviations: PK-i, interferon-induced protein kinase; eIF-2, eukaryotic initiation factor 2; dsRNA, double-stranded RNA; 2′-PDi, interferon-induced phosphodiesterase; NP40, Nonidet P40; VSV, vesicular stomatitis virus.

Measure of Phosphoprotein Phosphatase Dephosphorylating eIF-2. Phosphorylated eIF-2 was prepared by incubating (in 0.15 ml) 60 μg of eIF-2, 7 μg of PK-i [purified to step IV (15)], 0.33 mM [^{32}P]ATP (3.7 mCi/mmol), 0.4 μg of poly(rI)·(rC) per ml in 16 mM Hepes, pH 7.5/4 mM $MgCl_2$/96 mM KCl/0.8 mM dithiothreitol/8% glycerol for 30 min at 30°C. An aliquot of this reaction (4 μg of eIF-2) was incubated in 25 μl with 10 μl of cell extracts and 11 mM EDTA for 60 min at 30°C. Controls without cell extract were included. To check that EDTA blocks phosphorylation efficiently, non prephosphorylated eIF-2 was added in another set of tubes. Samples were processed by gel electrophoresis in sodium dodecyl sulfate as above.

Assay of Oligoisoadenylate Synthetase E. Cell extracts (25 μl) were mixed with 25 μl of packed poly(rI)·(rC)-Sepharose (26) for 5 min at room temperature, and the nonadsorbed material was removed by centrifugation for 2 min at 8000 × *g*. To the 25 μl of Sepharose pellet washed with Buffer B, 10 μl of 2.5 mM [α-^{32}P]ATP (0.3 Ci/mmol)/2.5 mM dithiothreitol was added. The suspension was incubated for 20 hr at 30°C, the supernatant was recovered by centrifugation, and the Sepharose was washed once with 10 μl of water. A 10-μl aliquot was treated with 5 μl of bacterial alkaline phosphatase (0.35 units dialyzed in 0.1 M Hepes, pH 7.5) in 30 mM Tris base for 60 min at 37°C. A 5-μl aliquot was electrophoresed on Whatman 3MM paper at 3000 V for 4 hr with AMP and ApA markers. After autoradiography the (2′-5′)ApA spots (R_F of 0.4 relative to 5′-AMP) were cut and assayed by scintillation counting.

Measure of Phosphodiesterase 2′-PDi. Reaction mixtures (10 μl) containing 6 μl of cell extracts and 1.6 mM (2′-5′)ApA in Buffer B were incubated for 60 min at 37°C. Aliquots (2 μl) were applied to thin-layer sheets of polyethyleneimine (Schleicher & Schuell) and chromatographed in 0.32 M LiCl. The 5′-AMP spots [R_F 0.25 relative to (2′-5′)ApA] were cut and eluted with 0.7 M $MgCl_2$ in 20 mM Tris·HCl (pH 7.5) and the absorbance was determined at 260 nm.

Materials. eIF-2 from rabbit reticulocyte, purified to near homogeneity, was a gift from D. Shafritz (New York). (2′-5′)-ApA was obtained from Y. Lapidot (Jerusalem). [^{32}P]ATP was purchased from the Radiochemical Centre (Amersham, England). Poly(rI)·(rC) was from P-L Biochemicals. Anti-mouse interferon serum was obtained from G. Galasso (National Institutes of Health).

RESULTS

Time Course of Protein Kinase PK-i Induction by Interferon. We have described the purification from interferon-treated L cells of protein kinase PK-i, which phosphorylates the small 35,000-M_r subunit of initiation factor eIF-2 (13, 15). The increased dsRNA-dependent phosphorylation of an endogenous polypeptide of 67,000-M_r in mouse cell extracts (5–13) and 69,000-M_r in human cell extracts (27), also seen in ^{32}P-labeled intact human and mouse cells after interferon and dsRNA treatment (ref. 28; unpublished results), has been tentatively attributed to an increase in this protein kinase. But, to have a true measure of the level of the protein kinase activity in cell extracts, it is necessary to use an exogenous substrate. We, therefore, developed a microassay for the phosphorylation of pure reticulocyte eIF-2 by extracts of L cells. The assay is based (*i*) on the specificity of protein kinase PK-i for the 35,000-M_r subunit of eIF-2 and (*ii*) on the fact that PK-i is activated by preincubation with dsRNA and ATP. Requirement for low concentrations of dsRNA distinguishes PK-i from the hemin-regulated eIF-2 kinase (29).

Cytoplasmic extracts, from as little as 25,000 cells lysed with the nonionic detergent NP40, were first preincubated with dsRNA and unlabeled ATP to activate PK-i, before adding eIF-2 and [γ-^{32}P]ATP. Exogenous eIF-2 added as substrate was the major phosphorylated product (Fig. 1*A*). Labeling of the 50,000-M_r subunit of eIF-2 was due to a contaminating protein kinase activity in the eIF-2 preparation used (see Fig. 1*B*, lane 3). Most of the endogenous substrates for the various protein kinases in the cell extracts were phosphorylated with nonradioactive phosphate during the preincubation and, therefore, did not appear on the autoradiographs (with the exception of a 100,000-M_r band). The absence of phosphorylation of the 67,000-M_r protein also resulted from the preincubation.

Monolayer cultures of L cells were exposed for various times to mouse interferon, and control cultures were submitted to the same changes of medium. The establishment of the antiviral state was measured by infecting duplicates of the cultures with vesicular stomatitis virus (VSV) at 0.1 plaque-forming unit per cell and determining the virus titer after 24 hr. From 8.4 log plaque-forming units/ml in untreated cultures, the virus titer was reduced by 2.8 log after 5 hr of interferon treatment and by 4.4 log after 24 hr of treatment.

The levels of protein kinase PK-i activity at various times after the addition of interferon are shown in Fig. 2. The activity began to rise at 3 hr, was increased 2-fold at 5 hr, and further increased progressively to over 5-fold at 24 hr. There was a 2-hr lag period after interferon addition during which no change in PK-i was observed. The level of PK-i in the control cultures was stable. For each point, two amounts of NP40 extracts (from 2 and 4 × 10^4 cells) were used to verify that the area of the eIF-2 phosphorylated band on the autoradiographs was measured under nonsaturating conditions. Table 1 shows the levels of protein kinase activity 8 hr after treatment with various con-

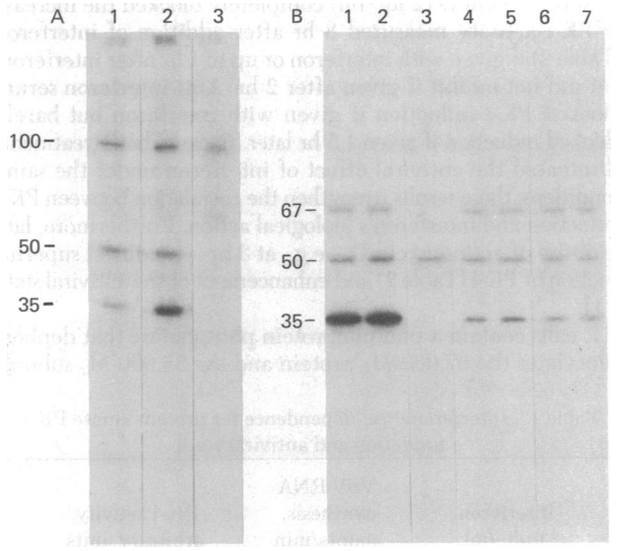

FIG. 1. Assay of protein kinase PK-i and of phosphoprotein phosphatase. (*A*) Protein kinase. NP40 extract (4 μl) was preincubated with unlabeled ATP and poly(rI)·(rC) before adding eIF-2 and [γ^{32}P]ATP. Lanes: 1, extract from untreated cells; 2, extract from cells treated with 200 units of interferon per ml for 24 hr; 3, same as 2, but eIF-2 was omitted. (*B*) Phosphoprotein phosphatase. Lanes: 1, eIF-2 phosphorylated with [γ-^{32}P]ATP and purified PK-i; 2, same as 1 but incubated for 60 min with EDTA; 3, same as 1 but PK-i was omitted in the phosphorylation step; 4 and 6, same as 2, but extract of control L cells was added; 5 and 7, same as 2, but extract of interferon-treated L cells was added. Interferon treatment (200 units/ml) was for 8 hr (lane 5) or 24 hr (lane 7); control cultures after 8 hr (lane 4) or 24 hr (lane 6). The positions of phosphorylated proteins 67,000-M_r (co-purified with PK-i), 50,000-M_r (eIF-2 middle subunit whose phosphorylation does not depend on PK-i), and 35,000-M_r (eIF-2 small subunit phosphorylated by PK-i) is shown.

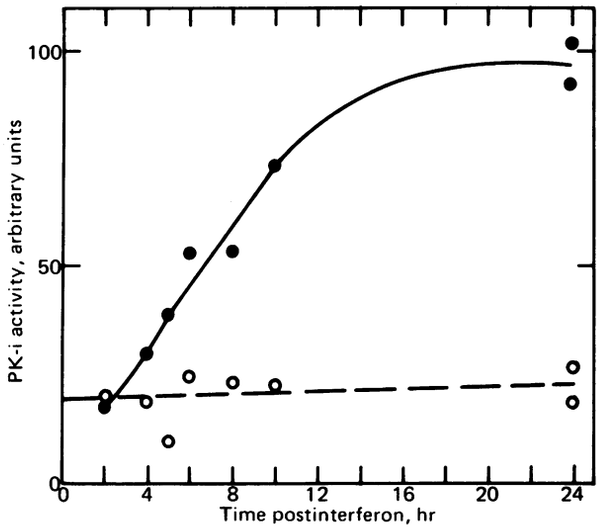

FIG. 2. Time course of protein kinase PK-i induction by interferon. Reaction mixtures contained 2 μl of NP40 extracts from L cells treated with 200 units of interferon per ml (●) or untreated cells (○). PK-i activity is expressed as the area (in mm^2) of the 35,000-M_r phosphorylated bands from scans of autoradiographs as shown in Fig. 1*A*.

centrations of interferon. For this experiment, 25,000 L929 cells in individual wells of a microtiter plate were used for each interferon dilution. In separate wells, the residual VSV RNA synthesis was measured. There is a clear correlation between the reduction of virus replication and the level of PK-i activity.

Actinomycin D (2 μg/ml) completely blocked the increase in PK-i activity measured 8 hr after addition of interferon (Table 2) if given with interferon or up to 1 hr after interferon, but did not inhibit if given after 2 hr. Anti-interferon serum blocked PK-i induction if given with interferon but barely blocked induction if given 1.5 hr later. Because both treatments eliminated the antiviral effect of interferon under the same conditions, these results strengthen the correlation between PK-i induction and interferon's biological action. Furthermore, late addition of actinomycin D—e.g., at 3 hr—produced superinduction of PK-i (Table 2) and enhancement of the antiviral state (31).

L cells contain a phosphoprotein phosphatase that dephosphorylates the 67,000-M_r protein and the 35,000-M_r subunit

Table 1. Interferon dose-dependence for protein kinase PK-i induction and antiviral state

Interferon, units/ml	VSV RNA synthesis, counts/min	PK-i activity, arbitrary units
0	1450	15
0.4	1310	13
2	350	35
10	90	80
50	0	100

L cells (line L929) in a 96-well microplate were exposed for 8 hr to the indicated concentrations of interferon, and NP40 extracts were assayed for PK-i activity as described in *Materials and Methods* and Fig. 2. To measure the antiviral state, cells in other wells were infected with VSV. One hour later the cells were labeled for 7 hr with [^{3}H]-uridine (45 Ci/mmol, 0.01 mCi/ml) in the presence of 2 μg of actinomycin D per ml, and viral RNA synthesis was measured as described (30). RNA synthesis without virus infection (150 counts per min) was subtracted.

Table 2. Effect of actinomycin D and anti-interferon serum added at various times after interferon on enzyme induction

Inhibitor added	Time after interferon addition, hr	Enzyme induction by interferon* PK-i	Enzyme induction by interferon* Oligoisoadenylate synthetase E
Actinomycin D at:	−2	0	0
	0	6	20
	1	21	29
	2	98	100
	3	160	145
	6	97	133
Control without inhibitor	—	100	100
Anti-interferon serum at:	0	2	7
	1.5	77	37
	3	80	84
Control without inhibitor	—	100	100

PK-i induction in L929 was measured as in Table 1 and Fig. 2. Oligoisoadenylate synthetase E induction in the same cell extracts was measured as in Fig. 3. Actinomycin D at 2 μg/ml or anti-mouse-interferon serum was added at the indicated times relative to interferon, and enzyme increase was measured 8 hr after interferon addition. The controls without inhibitor (100%) represent a 3-fold increase in eIF-2 phosphorylation and a synthesis of 12,000 cpm or 30 pmol of 2′-5′$(Ap)_nA$ cores for 10 μl of extract (a nucleotide synthesis without interferon of 2300 cpm was subtracted).
* % of control without inhibitor.

of eIF-2 (9, 15). Upon incubation of prephosphorylated eIF-2 in the cell extracts, specific dephosphorylation of the 35,000-M_r subunit was clearly observed (Fig. 1*B*), but this phosphatase activity was not changed by interferon treatment. The increased phosphorylation of eIF-2 in Fig. 2 cannot, therefore, be explained by a decreased dephosphorylation and must represent a true increase in PK-i activity.

Time Course of Oligoisoadenylate Synthetase E Induction by Interferon. This enzyme, which slowly polymerizes ATP into a series of oligonucleotides with the general structure ppp(A2′p)$_n$5′A, was previously isolated from interferon-treated cells by binding to poly(rI)·(rC)-Sepharose (18) or by purification through DEAE- and phosphocellulose (13, 23). When isolated by the latter procedure, enzyme E is dsRNA-dependent (13) and sediments on a glycerol gradient as a protein of 55,000–65,000 daltons (unpublished data). Because crude cell extracts contain a specific 2′-phosphodiesterase that degrades the oligoisoadenylate formed (ref. 23; see below), we could not assay directly oligoisoadenylate synthetase E in the NP40 extracts. Binding to poly(rI)·(rC) was, therefore, used and we measured incorporation of [α-^{32}P]ATP into the phosphatase-resistant cores (2′-5′)ApA and (2′-5′)ApApA separated by paper electrophoresis at pH 3.5 (13, 23) (Fig. 3*A*). Use of NP40 yielded much more activity than mechanical disruption, suggesting that enzyme E may be membrane-bound. The technique was used to measure the time course of enzyme E induction in monkey kidney cells BSC-1 (Fig. 3*A*) and in mouse L cells (Fig. 3*B*) treated by their homologous interferon. Increased enzyme E activity was clear in both cases after 10–12 hr. In the monkey cells, an increase in enzyme E activity could be detected much earlier also, and a decrease was seen after 24 hr. In L cells, the increase started only after 6 hr and was maximal (15-fold) at 24 hr. In these cells, the elevation of oligoisoadenylate synthetase E activity appears, therefore, later than that of protein kinase PK-i in the same extracts. The induction of enzyme E also remained sensitive to anti-interferon serum for a longer time than did induction of PK-i and was still inhibited over 60% if the antibodies were added 1.5 hr after interferon (Table 2). This

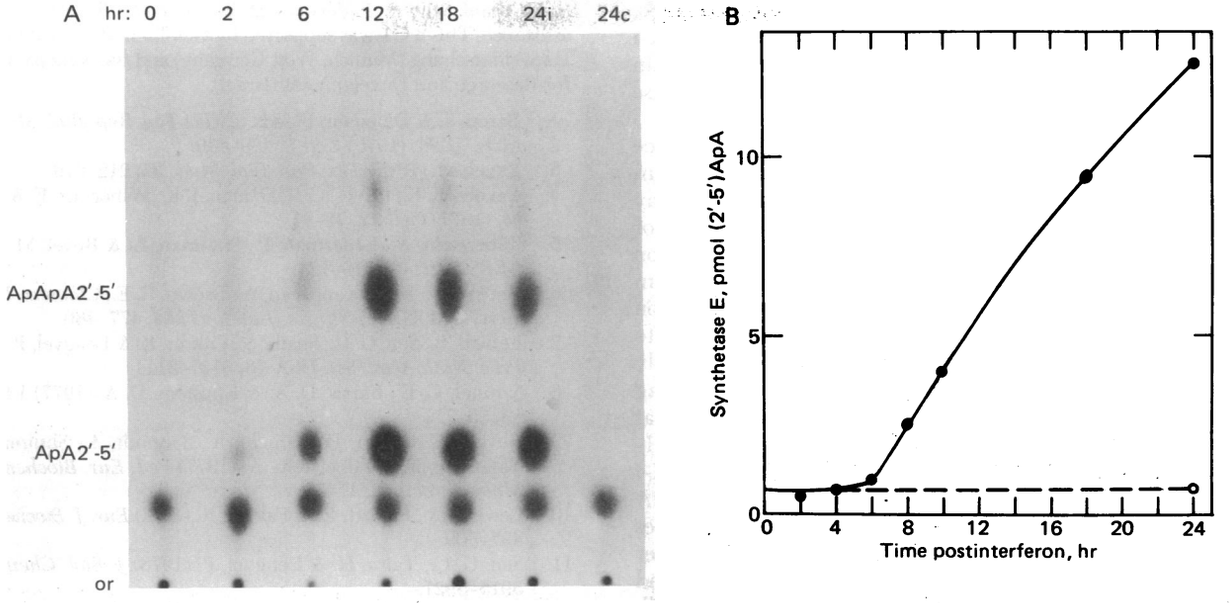

FIG. 3. Time course of oligoisoadenylate synthetase E induction by interferon. (*A*) Monolayers of BSC-1 cells in 60-mm plates were infected with simian virus 40 (25 plaque-forming units per cell) and treated 24 hr later by monkey interferon (100 units/ml). At the indicated time, NP40 extracts were prepared and oligoisoadenylate synthetase E activity was measured. Autoradiography of a gel of the bacterial alkaline phosphatase-treated products is shown. The position of (2'-5')ApA and (2'-5')ApApA was determined with synthetic markers. A control culture without interferon was analyzed 24 hr after mock treatment (lane 24c). (*B*) Noninfected L cells were treated with mouse interferon, NP40 extracts were prepared, and oligoisoadenylate synthetase E was measured (●). ○, Untreated cells. Results were for 1 μl of extract.

also indicates that interferon remains for some time accessible to the antibodies. Actinomycin D given up to 1 hr after interferon inhibited enzyme E induction but later produced superinduction as seen for PK-i (Table 2). In contrast to (2'-5')pppApApA synthetase E, the activity of ribonuclease F, which depends on the oligonucleotide, is not markedly increased in L cells after interferon treatment (23).

Increase in Phosphodiesterase 2'-PDi after Interferon Treatment. In a previous work, we reported the isolation from L cells of an enzyme that degrades oligoisoadenylate molecules (2'-5') pppApApA into ATP and 5'-AMP (23). This enzyme (2'-PDi) is a phosphodiesterase which is generally more active on 2'-5' than on 3'-5' dinucleoside monophosphates; its purification and properties will be reported in detail elsewhere (24). The enzyme is a 2'-phosphodiesterase characterized by its ability to cleave the 2' phosphate bond of many dinucleoside monophosphates; it is, for example, more active on (2'-5')ApA than on the 3'-5' isomer. This specific property allows us to assay for 2'-PDi in crude extracts, without interference by other ribonucleases, by using (2'-5')ApA as substrate and measuring the release of 5'-AMP. The activity was measured in extracts prepared by NP40 or better by mechanical disruption from L cell monolayers, under conditions of excess substrate, so that only 20% of the (2'-5')ApA was degraded. Under these conditions it is possible to observe an increase in the specific 2' phosphodiesterase after interferon treatment. Fig. 4 shows that the increase is maximal (3-fold) at 8 hr and decreases again later. Because the biological activity of (2'-5')pppApApA can also be destroyed by other enzymes in crude extracts (such as, for example, by phosphomonoesterases), assay of the biological half-life of the RNase F effector may fail to show the increase in 2'-PDi activity (32). With 2'-5' dinucleoside monophosphates, 4- to 5-fold increases in 2'-PDi after interferon treatment were seen in L929 and CCL1 cells (24).

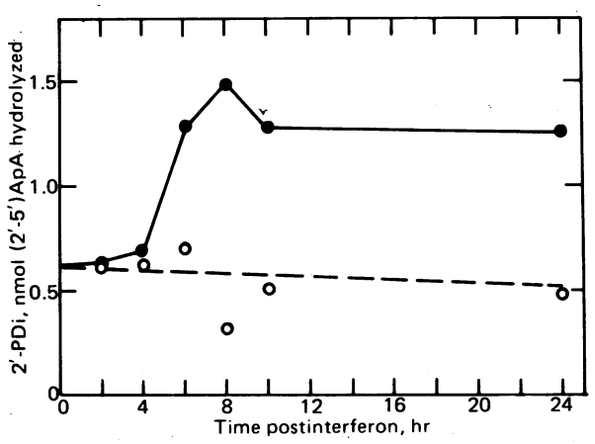

FIG. 4. Time course of phosphodiesterase 2'PDi induction by interferon. Conditions for the assay are detailed in *Materials and Methods*. The hydrolysis of (2'-5')ApA into 5'-AMP and adenosine was measured in NP40 extracts prepared at various times after treatment of L cells with 200 units of interferon per ml (●) or in untreated cultures (○).

DISCUSSION

Studies of cell-free protein synthesis systems prepared from interferon-treated cells have led during the past year to the isolation of two dsRNA-dependent enzymes, eIF-2 protein kinase PK-i and oligoisoadenylate synthetase E (13, 14). In the present work, we show that quantitative measurements of the level of these enzyme activities can serve as a model to study interferon's action on the cell. Measurements can readily be made on cytoplasmic extracts prepared by a simple detergent treatment from 2–4 × 10^4 cells. Monolayer microcultures from mouse, monkey, or human cells can be used. The data presented show, in mouse L cells, how these enzyme activities rise after addition of interferon. The dsRNA-dependent protein kinase PK-i, phosphorylating eIF-2's small subunit, is increased already after 3–4 hr. Induction of PK-i becomes visible with the smallest concentrations of interferon (2 units/ml) that reduces by over 50% viral RNA synthesis in VSV-infected cells. Interferon can produce a 6- to 7-fold increase in the eIF-2 protein kinase over

the level seen in untreated cells. Whether, in untreated cells, eIF-2 phosphorylation is due to the same protein kinase PK-i remains to be examined. In L cells, a 10- to 15-fold increase in oligoisoadenylate synthetase E is demonstrable, but the increase starts at 4–6 hr after interferon.

An important feature of these kinetic studies is the existence of a lag period between addition of interferon and enzyme increase. Although the longer lag in L929 cells for enzyme E may be an artifact of the assay, it is interesting that induction of enzyme E seems to require a prolonged interaction of interferon with the cell, as shown by the fact that anti-interferon serum still partially inhibits induction when given 1.5 hr after addition of interferon to the culture. PK-i induction is less sensitive to delayed addition of the antibodies, although it is completely inhibited if these are given together with interferon. The lag period may be also studied by addition of actinomycin D at various times after interferon. When added during the first 1- to 2-hr lag period, actinomycin D prevents the increase in PK-i and synthetase E activities, suggesting that an early DNA transcription event is needed for induction of these enzymes as well as for the establishment of the antiviral state (33). After the lag period, induction becomes actinomycin D-resistant, and there is a progressive increase in PK-i and E activity per cell, suggesting accumulation of these enzymes. Later, actinomycin D even produces some superinduction of the enzymes, as also shown for the antiviral state (31). Whether or not there is *de novo* synthesis of the dsRNA-dependent enzymes PK-i and E, or one of their components, is not yet clear, but cycloheximide inhibits induction (unpublished results).

Similar studies can be done in other cells. In monkey kidney cells treated by monkey interferon 24 hr after infection by simian virus 40, the increase in oligoisoadenylate synthetase E was demonstrated and appears to start earlier than in L929 cells—i.e., 2 hr after interferon. This system is interesting to study because, at the time the enzyme measurements are made, interferon-induced selective inhibition of simian virus 40 T-antigen and capsid protein mRNA translation (most cellular protein synthesis is not inhibited) can be demonstrated (4). Preliminary evidence for increased eIF-2 protein kinase was also obtained in these virus-infected monkey cells. We have been able to use the same methods to measure the induction of protein kinase PK-i and synthetase E in human diploid foreskin fibroblast treated with their own interferon. In these cells, actinomycin D still partially inhibited induction even when added as late as 3 hr after interferon (unpublished results).

We describe here the increase of a third enzyme activity after interferon treatment of L cells. This enzyme is a specific phosphodiesterase 2′-PDi, which cleaves the 2′ phosphate bond of synthetic nucleotides and of (2′-5′)pppApApA (23). Its increase may regulate the amount of oligonucleotide and prevent excessive activation of RNase F, which could damage the cells. On the other hand, we found that this phosphodiesterase degrades also the CpCpA terminus of tRNA (24). In cell-free systems, it produces a tRNA-reversible inhibition of protein synthesis in the absence of dsRNA, similar to that observed in extracts of interferon-treated L cells (34). The three enzymes studied alter, in different ways, the cell's protein synthesis machinery and have different requirements for dsRNA. Their functions in the antiviral and anticellular effects of interferon are not fully established. The ability to measure the induction of these specific enzymes within a few hours of interferon addition should, however, become an important tool to investigate the early steps of interferon action.

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