INHIBITION OF PROTEIN SYNTHESIS IN NONINFECTED L CELLS BY PARTIALLY PURIFIED INTERFERON PREPARATIONS

TERRY C. JOHNSON, MICHAEL P. LERNER, and GERALD J. LANCZ

From the Department of Microbiology, Northwestern University, School of Medicine, Chicago, Illinois 60611

ABSTRACT

Partially purified interferon preparations, obtained from L-cell monolayers infected with Newcastle disease virus (NDV), were shown to inhibit protein synthesis in noninfected L cells. The incorporation of several amino acids-^{14}C was equally sensitive to the pretreatment of the cells with the interferon preparation. Treatment of L-cell monolayers for 24 hr with 800 units of interferon resulted in a 50% decrease in amino acid incorporation. The degree of inhibition was found to be a function of the interferon concentration and the time of exposure of the cells to the partially purified preparations. No inhibitory effect was detected in medium obtained from noninfected cells and purified in an identical manner. The inhibitory effect was shown to be cell specific in that the partially purified interferon from L cells did not reduce amino acid incorporation in heterospecific cell lines. Heating the interferon preparations at 60°C destroyed their antiviral activity and their ability to inhibit valine-^{14}C incorporation in L cells.

Several investigators have previously reported that crude and partially purified interferon preparations exert little, if any, effect on the metabolism of the noninfected cell (8, 10, 14, 19). Other workers have found that crude and semipurified interferon preparations did produce an inhibitory effect on cell metabolism or mitosis (1, 3, 11, 16, 18). In some of the latter cases it was suggested that the inhibition was the result of another component and not the interferon itself (1, 4, 11). Few reports have appeared comparing the metabolic inhibitory action to the antiviral action of the interferon preparations. The determination of the effects of these preparations on the metabolism of normal, noninfected host cells may lead to a greater understanding of the relationship between host-cell directed proteins produced during interferon induction. This paper reports a study of the effects of partially purified interferon on noninfected L cells. The kinetics of the inhibitory action and its relationship to the antiviral activity of the preparations are reported.

MATERIALS AND METHODS

Cells and Viruses

Mouse L-fibroblasts, HeLa cells, Chinese hamster (CH) cells, baby hamster kidney (BHK) cells, and primary chick embryo cells were all grown as monolayers in 32-oz prescription bottles containing Eagle's medium (5) in Hanks' (6) balanced salt solution (BSS) supplemented with 10% whole calf serum, 100 units of penicillin, 100 μg of streptomycin, and 50 μg of neomycin sulfate per milliliter.

Pools of Newcastle disease virus (NDV), Roakin strain, were prepared by inoculating 10–11-day-old embryonated chick eggs and harvesting the allantoic fluid approximately 48 hr after infection. The resulting NDV pools were titrated by plaque assay on

primary chick-embryo cell monolayers and were found to contain 2–4 × 10^9 plaque-forming units (pfu) per milliliter. Mengovirus pools were prepared by infection of L-cell monolayers.

Interferon

PRODUCTION: Interferon was produced by infecting L-cell monolayers with NDV at a multiplicity of 10–20 pfu/cell. Following an adsorption period of 60 min at 37°C, the monolayers were washed two to three times with warm BSS for removal of unadsorbed virus and allantoic fluid. Approximately 75 ml of double strength Eagle's medium (2 parts amino acids, vitamins, and glutamine), without serum, were added to each culture, and the infected cell monolayers were incubated for 48 hr at 37°C.

PURIFICATION: After incubation, the crude interferon medium was collected, and the NDV was inactivated overnight at pH 2.0 (4°C). The inactivated preparation was clarified by centrifugation at 100,000 *g* for 2 hr. The resulting supernatant fluid was treated with perchloric acid, and the interferon was precipitated with zinc acetate essentially as described by Lampson et al. (9). The final interferon precipitate was dissolved in 0.2 N HCl and dialyzed at 4°C for 48 hr against 0.15 M NaCl. The small amount of insoluble material (denatured protein) was removed by centrifugation at 3,000 *g* for 1 hr, and the final supernatant fluid was designated as the partially purified interferon. An undiluted sample of this interferon preparation, assayed on primary chick-embryo monolayers, revealed no infectious virus.

INTERFERON ASSAY: Interferon was titered by a plaque-reduction assay with mengovirus and L-cell monolayers by pretreating the cells for 18–24 hr with serial dilutions of the purified preparations. A unit of interferon was designated as the amount of interferon that produced a 50% reduction in viral plaques. The specific activities of the partially purified interferon preparations that were employed in these studies ranged from 130,000 to 190,000 (units of interferon per milligram protein). Interferon preparations were stored at −70°C.

^{14}C-Labeled Compounds

Uniformly labeled L-isoleucine (222 mc/mmole), L-leucine (222 mc/mmole), L-lysine (223 mc/mmole), L-phenylalanine (334 mc/mmole), L-threonine (148 mc/mmole), L-tyrosine (375 mc/mmole), and L-valine (185 mc/mmole) were purchased from New England Nuclear Corp., Boston, Mass.

Cellular Protein Synthesis

Cell monolayers, grown in 2-oz French square bottles (Arthur H. Thomas Co., Philadelphia, Pa.), were rinsed with BSS and fed with 2.5 ml of Eagle's medium supplemented with 10% calf serum and containing various concentrations of purified interferon. The cells were incubated at 37°C with interferon as described in the following experiments. In each experiment identically treated cells, as controls, were incubated with medium which had been obtained from noninfected L-cell monolayers and which had been purified by the procedure described for interferon preparations. After incubation, the medium was decanted, and the monolayers were carefully rinsed twice with warm BSS. Protein was labeled by adding 1 ml of Eagle's medium (minus the amino acid of interest) containing 2% dialyzed calf serum and 0.25 or 1.0 μc of the amino acid-^{14}C. Unless otherwise stated, the preparations were incubated at 37°C for 60 min. After incubation, the cellular incorporation of amino acids was terminated by the addition of 5.0 ml of cold BSS. The monolayers were rinsed two to three additional times with 10.0 ml of cold BSS for removal of exogenous, labeled amino acid.

Cell protein was extracted by dissolving the cells in 0.15 M NaCl and three drops of 1 N NaOH followed by repeated precipitation with hot 5% trichloroacetic acid (17). The resulting protein precipitates were collected on nitrocellulose membrane filters and then dried; the radioactivity was measured in a Nuclear Chicago gas flow counter. The amount of radioactivity in the acid-insoluble protein fractions is reported as the counts per minute per milligram of cellular protein (specific activity).

Protein concentrations were determined by the method of Lowry et al. (12), with crystalline bovine serum albumin as the protein standard.

RESULTS

Two sets of L-cell monolayers were employed for determination of the effect of partially purified interferon on cellular protein synthesis. One set of cultures was fed Eagle's medium containing a 10^2 dilution of partially purified interferon (640 units ml). A second set, as a control, was fed Eagle's medium containing a 10^2 dilution of medium purified from noninfected L cells (no interferon activity). The cells were incubated for 16 or 24 hr at 37°C, and then both sets were pulsed with 0.25 μc of valine-^{14}C for 60 min as described in Materials and Methods. As is seen in Table I, prior incubation of L-cell monolayers with the diluted interferon markedly inhibited cellular incorporation of valine-^{14}C into acid-insoluble proteins. However, there were no significant changes in the amount of radioactive amino acid in the acid-soluble intracellular pools of control and interferon-treated cells. Incubation of L cells with the control preparations, obtained

TABLE I

Valine-^{14}C Incorporation by Treated and Nontreated L Cells

	Time preincubated	Interferon	Acid-soluble (pool)	Acid-insoluble (protein)
	hr	*units/ml*	*cpm/mg protein*	
Experiment 1	16	control	1,760	19,100
	16	640	1,710	12,400
	16	640	1,880	13,200
Experiment 2	24	control	1,610	20,500
	24	640	1,400	11,800
	24	640	1,490	10,700

L-cell monolayers were preincubated with 640 units of interferon per milliliter (1/100 dilution) for either 16 or 24 hr. After pretreatment, the cells were pulsed with 0.25 μc of valine-^{14}C for 60 min as described in Materials and Methods. The radioactivity in the intracellular acid-soluble pool was determined by counting an aliquot of the soluble material after the first trichloroacetic acid precipitation.

from noninfected cultures, had no inhibitory effect on protein synthesis, and the results were always similar to those obtained when L cells were incubated with Eagle's medium alone.

Various concentrations of the interferon preparations were incubated on L-cell monolayers for 24 hr to measure their effect on cellular protein synthesis. After incubation the treated and control cells were pulsed with either valine-^{14}C or lysine-^{14}C. The cells treated with the highest concentrations of interferon exhibited the most striking inhibition of protein synthesis (Table II). The degree of inhibition is seen to vary with the interferon concentration employed, although the addition of 800 units of interferon per milliliter seemed to saturate the system since doubling the concentration did not result in a greater inhibition. In addition, both valine-^{14}C and lysine-^{14}C incorporations were equally sensitive to pretreatment with interferon.

Fig. 1 shows the kinetics of valine-^{14}C incorporation by treated and control cells during the routine 60 min pulse. L-cell monolayers were incubated with and without interferon for 36 hr, and then the cells were pulsed with 0.25 μc of valine-^{14}C. The cells that had been previously exposed to 800 units of interferon per milliliter for 36 hr exhibited a lower rate of protein synthesis throughout the 60 min incubation period (Fig. 1).

The preceding data suggests that the inhibition of cellular protein synthesis depends on the time of preincubation of the cells with the interferon preparations. Fig. 2 shows the data resulting from the exposure of L-cell monolayers to partially purified interferon (800 units ml) for time periods

TABLE II

Effect of Interferon Concentration on Amino Acid-^{14}C Incorporation by L Cells

	Amio Acid-^{14}C	Interferon*	Protein	Control
		units/ml	*cpm/mg*	%
Experiment 1‡	valine	control	76,000	100
		80	62,000	82
		800	35,000	46
Experiment 2‡	lysine	control	32,500	100
		80	23,000	72
		800	17,500	54
Experiment 3§	valine	control	24,700	100
		16	23,900	95
		200	21,600	89
		400	18,100	72
		800	13,900	56
		1600	13,600	55

* L-cell monolayers were preincubated for 24 hr with the appropriate concentration of interferon.

‡ 1.0 μc of amino acid-^{14}C was employed.

§ 0.25 μc of amino acid-^{14}C was employed.

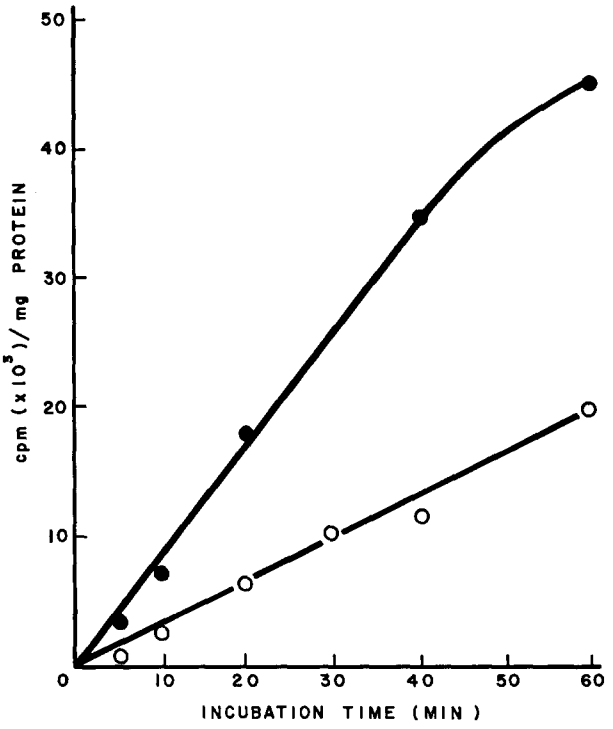

FIGURE 1 Rate of valine-^{14}C incorporation by treated and control L cells. Cells were pretreated with 800 units of interferon for 36 hr and were pulsed with 0.25 μc of valine-^{14}C. ●, control cells; ○, interferon-treated cells.

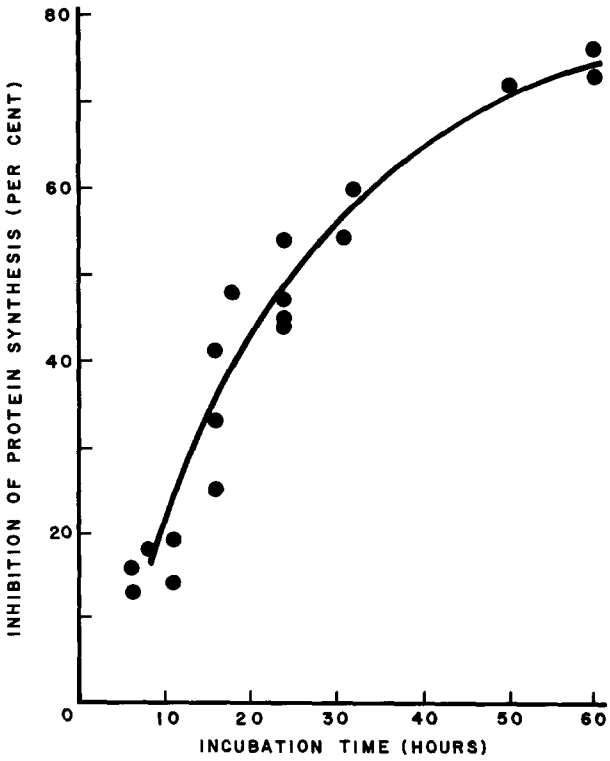

FIGURE 2 Effect of interferon preparations on valine-^{14}C incorporation by L-cell monolayers. Cells were exposed to the interferon preparations (800 units/ml) for various lengths of time and then pulsed with valine-^{14}C as described in the text.

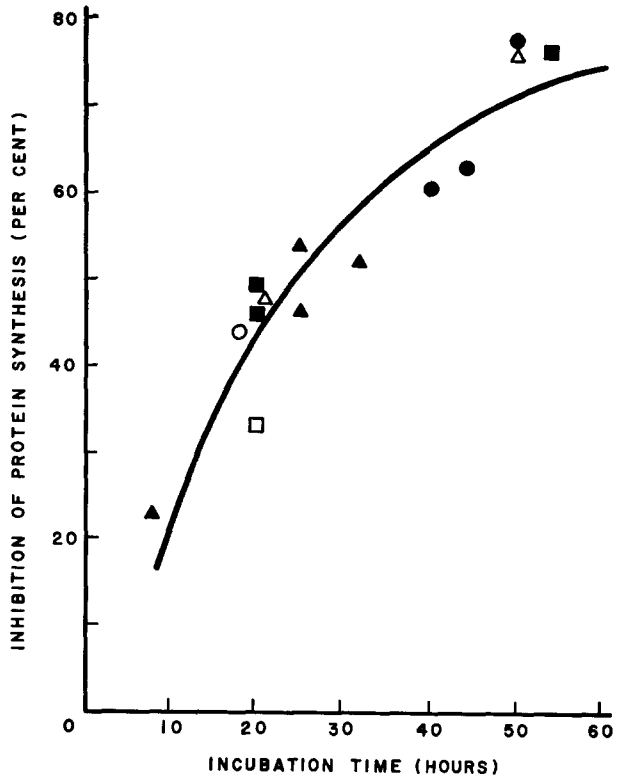

FIGURE 3 Effect of interferon preparations on the incorporation of several amino acids-^{14}C by L-cell monolayers. Cells were pre-incubated with partially purified interferon preparations (800 units/ml) for various lengths of time and then were pulsed with the different amino acids as described in the test. The solid line represents the inhibition of valine-^{14}C incorporation. ●, isoleucine; ■, tyrosine; ▲, lysine; ○, phenylalanine; □, leucine; △, threonine.

ot 6–60 hr. Cellular protein synthesis was found to be increasingly sensitive as the time of exposure was increased. By 60 hr the ability of L cells to incorporate valine-^{14}C into acid-insoluble proteins was reduced by nearly 80%.

Fig. 3 shows the results obtained when several different amino acids-^{14}C were employed to measure the effect of interferon preparations on L-cell protein synthesis. The incorporation of all amino acids tested was sensitive to prior treatment of the cells with the interferon preparations. In addition, the degree of inhibition, as measured with the various amino acids, was very similar to the rate established for valine incorporation (solid line).

Since the antiviral action of interferon is specific for the species in which it is produced, the ability of L-cell interferon to inhibit protein synthesis in other tissue culture cells was determined. Table III shows that pretreatment of HeLa, BHK, CH and primary chick-embryo cells with 800 units of interferon for 24 hr did not significantly effect protein synthesis. The specificity of the interferon preparation was maintained even after pretreatment of HeLa and chick embryo cells with 1,600 units of interferon for 50 hr, although, during this same time period, L-cell protein synthesis was reduced by approximately 70%.

The antiviral activity of interferon has been shown to be destroyed by heat (14, 19). The effects of heating L-cell interferon preparations at 60°C are shown in Table IV. Heating for 15 and 30 min completely destroyed the ability of the interferon preparations to inhibit L-cell protein synthesis. In addition, over 99% of the interferon activity, as determined by the plaque-reduction technique, was abolished.

In the course of these studies the interferon preparations stored at −70°C gradually lost their antiviral activity. This finding is in agreement with previous reports that purified mouse interferon is unstable at this low temperature (14). In each case, a loss in interferon (antiviral) titer also resulted in a loss in the ability of the preparations to inhibit protein synthesis in L cells.

DISCUSSION

The results here show that partially purified L-cell interferon preparations exert an inhibitory

TABLE III

Specificity of Interferon Preparations on Valine-^{14}C Incorporation

Cell line	Time preincubated	Interferon	Protein	Control
	hr	*units/ml*	*cpm/mg*	%
L cell*	24	control	76,000	100
L cell	24	800	35,000	46
HeLa*	24	control	84,000	100
HeLa	24	800	78,000	93
BHK‡	24	control	22,100	100
BHK	24	800	22,600	102
CH‡	24	control	26,200	100
CH	24	800	24,500	94
L cell*	50	control	77,000	100
L cell	50	1600	21,000	28
Chick embryo*	50	control	44,000	100
Chick embryo	50	1600	54,000	116
HeLa*	50	control	39,800	100
HeLa	50	1600	39,000	98

* 1.0 μc of valine-^{14}C was employed.
‡ 0.25 μc of valine-^{14}C was employed.

effect on protein synthesis in normal, noninfected L cells. No significant alterations in the acid-soluble intracellular amino acid pools of treated and control cells were detected. This indicates that pretreatment with interferon preparations did not grossly effect cellular permeability to the ^{14}C-amino acid. After incubation of L-cell monolayers with 800 units of interferon for 36 hr, a marked inhibition of valine-^{14}C incorporation was detected throughout a 60 min pulse (Fig. 1). In addition, when medium was obtained from noninfected L-cell monolayers and purified in a manner identical with that used for the interferon preparations, no inhibition of cellular protein synthesis was observed. This clearly illustrates that the inhibitory substance was produced as a result of the infection of the host cells with NDV virus

Paucker et al. (16) reported that a single exposure of L cells to 700 units of interferon was capable of depressing cell growth. In addition, cultures of L cells that have ceased interferon production have been shown to be refractory to further stimulation of interferon production (15). Even pretreatment of L cells with interferon has been shown to depress the induction of interferon synthesis (15). The present data support these previous observations and indicate that the depression of cell growth and interferon production may be the result of an inhibition of cellular protein synthesis. The degree of this inhibition was proportional to the interferon concentration, although 800 units per milliliter seemed to produce a maximal effect in 24 hr. However, recent experiments in this laboratory have shown that more concentrated preparations exert their inhibitory effect faster.

The inhibition of valine-^{14}C incorporation was dependent upon the time of exposure of the L-cells to the interferon preparations. The degree of inhibition was seen to rapidly increase until approximately 60 hr of exposure by which time nearly 80% of the cellular protein synthesis was inhibited. The inhibition of amino acid incorporation was not restricted to valine. The incorporation of isoleucine, leucine, lysine, phenylalanine, threonine, and tyrosine was also affected to the same degree by pretreatment of L-cell monolayers with the interferon preparations. In addition, the inhibition observed with each of these amino acids followed the same kinetics (Fig. 3). Since the inhibition was not restricted to any particular amino acid, the data indicate that pretreatment of L cells with the partially purified interferon preparations produced an inhibition of over-all protein synthesis.

Since the antiviral activity of interferon is highly specific in its cellular host range, the ability

TABLE IV

Effect of Heat (60°C) on the Ability of Interferon Preparations to Inhibit Valine-^{14}C Incorporation by L Cells

Time of heating*	Protein	Control	Residual interferon activity
min	*cpm/mg*	%	*units/ml*
—	29,700 (control)	100	—
0	20,500	69	40,000
0	20,100	68	40,000
15	31,500	106	<400
15	32,000	108	<400
30	33,000	111	<400
30	29,400	99	<400

* An aliquot of interferon, containing 40,000 units, was heated in a water bath at 60°C. A sample was removed just before heating, and after 15 and 30 min at 60°C. A 1/100 dilution (400 units per milliliter) was then incubated on L-cell monolayers for 24 hr. In addition, each sample was titrated, by plaque reduction assay, for determination of the residual interferon activity.

of these partially purified preparations to inhibit protein synthesis in several cell lines was determined. Amino acid incorporation by HeLa, BHK, CH, and primary chick embryo cells was not affected by pretreatment of the cell lines with the L-cell interferon preparations. Even after pretreatment of HeLa and chick-embryo monolayers with 1,600 units of interferon per milliliter for 50 hr, which produced over a 70% loss of L-cell protein synthesis, no significant decrease in amino acid incorporation was observed.

Partially purified mouse interferon has been reported to be thermolabile (14, 19). In a further attempt to correlate the interferon activity with the ability of the preparations to inhibit protein synthesis, samples of interferon were heated at 60°C for 15 min. These samples had no effect on valine-^{14}C incorporation by L-cells. In addition, over 99% of the original antiviral activity was destroyed. The simultaneous loss of antiviral activity and inhibition of protein synthesis in the interferon preparations were also noted when the samples were stored for prolonged periods of time at −70°C. The thermolability of both the antiviral activity and the inhibition of protein synthesis suggests that the two functions are closely related, specific to L cells, and have no detectable influence on heterospecific cell lines.

Preliminary experiments indicate that these interferon preparations have little effect on RNA and ATP synthesis in L cells when the cultures are exposed to 800 units of interferon per milliliter for 24 hr. At the present time we have been unable to separate the antiviral activity from the protein synthesis inhibitory action by gel filtration and the use of DEAE-cellulose column chromatography.

Recently, investigators (2, 13) have demonstrated that interferon inhibits virus replication at the translational level. Although the inference is tempting, the data reported here do not show that the antiviral component and the inhibitor of protein synthesis are identical. In fact, even if interferon itself were responsible for the inhibition of protein synthesis in noninfected L cells, it does not necessarily follow that this phenomenon is related to interferon's mode of antiviral action. Our data, as well as those of several other investigators (10, 19, 20), indicate that the antiviral activity can be expressed without an appreciable effect on normal cellular metabolism. However, it is possible that the results presented here reflect a quantitative difference between the more resistant host metabolism and a more sensitive viral mechanism of translation.

The active component may be the result of a cellular response to virus infection, independent of interferon synthesis, that may be associated with the cell destruction without virus replication reported previously (7). If the inhibition of cellular protein synthesis is effected by a component other than interferon, we have been unable to separate this component from the antiviral component. In this respect, we have been unable to reconcile the fact that this protein inhibitor was found only after the induction of interferon synthesis, and that its action, like that of interferon, was mediated only in L cells.

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