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Oligonucleotide inhibitor of protein synthesis made in extracts of interferon-treated chick embryo cells: Comparison with the mouse low molecular weight inhibitor

(mechanism of interferon action/double-stranded RNA/avian cell-free systems)

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ABSTRACT **Cytoplasmic extracts of interferon-treated primary chick embryo cells contain an enzyme activity that synthesized an inhibitor of chick cell-free protein synthesis. The same activity was detected in extracts of cells treated with mock preparations of interferon, but at <0.3% of the level found in interferon-treated cell extracts. The enzyme was activated by double-stranded RNA and could be isolated by binding to columns of poly(I)·poly(C)-agarose. In the column-bound state, the enzyme reacted with ATP to synthesize the inhibitor, which could then be continuously eluted from the column. The inhibitor was purified and its structure and function were compared with those of the low molecular weight inhibitor of protein synthesis made by an enzyme from interferon-treated mouse L cells. The avian and mammalian inhibitors comigrated on thin layers of polyethyleneimine-cellulose during chromatography in three different solvent systems, and they coeluted as a series of peaks from columns of DEAE-cellulose during sodium chloride gradient elution. Digestion with bacterial alkaline phosphatase or snake venom phosphodiesterase yielded products that similarly comigrated. Functionally, the two inhibitors were interchangeable: both inhibited protein synthesis in extracts of mammalian and avian cells, producing 50% inhibition at a concentration of about 0.3 nM (AMP equivalents). We conclude that the chick cell-derived oligonucleotide inhibitor has a structure that is closely related or identical to that of the inhibitor made in the mouse system, and that both preparations inhibit cell-free protein synthesis in a non-species-specific manner.**

Interferon treatment of animal cells confers resistance to viral infection (1) by a process that involves cellular transcription and translation (2, 3). Although the mechanism by which viral replication is blocked remains unclear, several independent studies using cell-free extracts of interferon-treated mouse cells have provided evidence for an interferon-mediated inhibition of translation (4–7). Protein synthesis in extracts of interferon-treated cells also shows an enhanced sensitivity to inhibition by double-stranded RNA (dsRNA) (8–10). Furthermore, three new enzyme activities appear upon addition of dsRNA to extracts of interferon-treated mouse cells: a highly specific protein kinase (11–14), an endoribonuclease (15, 16), and an enzyme that synthesizes from ATP an oligonucleotide that is of unusual structure and is a potent inhibitor of cell-free protein synthesis (12, 17, 18). The interrelationships of these three enzyme activities and their relevance to the mechanism by which interferon blocks viral replication are unclear at present.

Almost all the work using cell-free systems from interferon-treated cells has been performed with extracts of continuous lines of mouse cells. Work with cell extracts from other sources has been limited because of difficulties in preparing active protein-synthesizing systems. However, we recently developed a procedure for preparing, from primary chick embryo cells, extracts that actively translate various exogenous mRNAs (19). Moreover, in response to purified vesicular stomatitis virus (VSV), these extracts execute coupled transcription and translation of the viral genes in reactions that continue for several hours (19). The chick cell extracts revealed an interferon-mediated inhibition of cell-free protein synthesis that was not due to an effect on mRNA synthesis, processing, or degradation (20). In the presence of low concentrations of dsRNA, the inhibition was accentuated and under these conditions an effect on the viral mRNA was not ruled out (20). This paper describes experiments that show that the inhibitory effect of dsRNA in the chick system is mediated by the synthesis of an oligonucleotide whose structure appears to be identical to that synthesized in the mouse L-cell system (17, 18) and that this oligonucleotide acts as an extremely potent inhibitor of cell-free protein synthesis.

MATERIALS AND METHODS

The preparation and assay of chick interferon, VSV, and cell-free extracts of primary chick embryo cells have been described (19, 20). Interferon was induced by infecting primary chick embryo cells with UV-irradiated Newcastle disease virus (20). It was partially purified by perchloric acid treatment, and the preparations used had specific activities of $1–2 \times 10^5$ units/mg. Mock interferon preparations were made in parallel from the medium of cells that had not been infected. Postribosomal supernatants were prepared from the cell extracts, after preincubation and gel filtration, by centrifugation at $100,000 \times g$ for 2 hr. They contained about 2 mg of protein per ml. The reaction conditions for coupled transcription and translation were as described (19, 21). For conventional cell-free protein synthesis in response to purified VSV mRNA, the incubation mixture contained: 20 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (Hepes) (pH 7.6); 33 mM NH_4Cl; 47 mM KCl; 2.5 mM Mg acetate; 0.2 mM spermidine; 1 mM ATP; 0.5 mM GTP, CTP, and UTP; 10 mM creatine phosphate; 80 μg of creatine phosphokinase per ml; 50 μM appropriate unlabeled amino acids; 1 mM dithiothreitol; reconstituted protein hydrolysates containing 15 ^3H- or ^{14}C-labeled L-amino acids (Schwarz/Mann, algal profile) at 100 μCi/ml (^3H) or 10 μCi/ml (^{14}C); VSV mRNA [synthesized *in vitro* and purified by oligo(dT)-cellulose chromatography (19)] at 3–5 μg/ml; and 20 or 30% cell-free extracts, to give a final protein concentration of about 1 mg/ml. Incubation was at 30° for 2 hr, and isotopic

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Abbreviations: dsRNA, double-stranded RNA; VSV, vesicular stomatitis virus; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

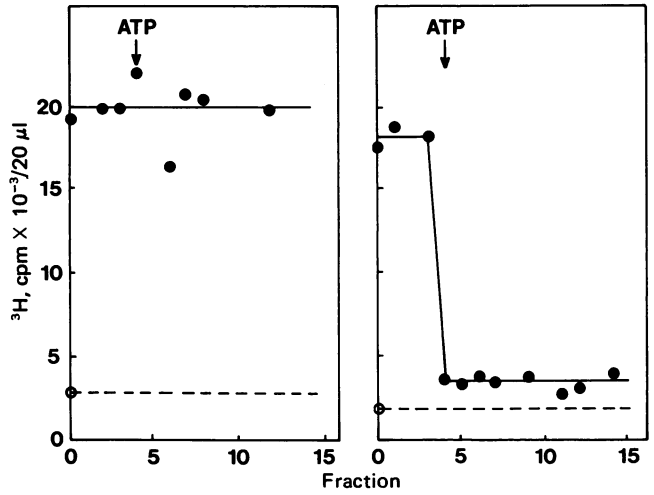

FIG. 1. Synthesis of the inhibitor by column-bound enzyme preparations. Columns of poly(I)·poly(C)-agarose (P-L Biochemicals; 250 µl) received 50 µl of postribosomal supernatants from mock-interferon-treated primary chick embryo cells (*Left*) or interferon-treated cells (250 PR_{50} units/ml for 17) (*Right*). The columns were incubated at 30° and washed extensively with 10 mM Hepes, pH 7.6/50 mM KCl/1.5 mM Mg acetate/7 mM 2-mercaptoethanol/20% glycerol. The flow was interrupted for 15 min between each 250-µl fraction. At the positions indicated, 1 mM ATP was added to the column buffers and the collection of fractions was continued. Fractions were assayed for their ability to inhibit protein synthesis after 1:10 dilution into cell-free extracts of untreated primary chick embryo cells programmed by VSV mRNA (19). ●, Incorporation of ^{3}H-labeled amino acids in the presence of VSV mRNA; ○, endogenous incorporation.

incorporation was monitored as described (21). References to the other methods used are in the text.

RESULTS

The interferon-mediated inhibition of protein synthesis observed in extracts of primary chick cells is enhanced by low concentrations of dsRNA (20). The factor(s) responsible reside in the soluble fraction of the cell because incubation of the postribosomal supernatant of interferon-treated cells with dsRNA and ATP resulted in the formation of a species that was able to inhibit protein synthesis profoundly in an extract of untreated chick cells, even after extensive dilution (Table 1). The interferon postribosomal supernatant, dsRNA, and ATP all were essential for the formation of the inhibitor in detectable amounts, and the inhibitor, once formed, was stable to heating at 90° for 10 min (Table 1). The conditions of synthesis and the heat-stability of the chick inhibitor were the same as those described for the interferon-treated mouse cell-derived inhibitor of translation (11, 12). In the latter case, affinity chromatography on columns of poly(I)·poly(C)-agarose was used successfully to isolate and partially purify the enzyme(s) responsible for inhibitor synthesis (17). Accordingly, the postribosomal supernatants from interferon-treated and mock-interferon-treated chick cells were applied to small columns of poly(I)·poly(C)-agarose, and the columns were washed extensively. The columns and any cellular material that remained bound to them were then incubated at 30° and the washing was continued. The eluate from neither column was inhibitory for chick cell-free protein synthesis unless ATP was present in the buffer perfusing the column (Fig. 1). Under these conditions, the column that had received the extract from interferon-treated cells generated a potent inhibitor that could be continuously eluted from the column as long as ATP was present in the perfusion buffer. In

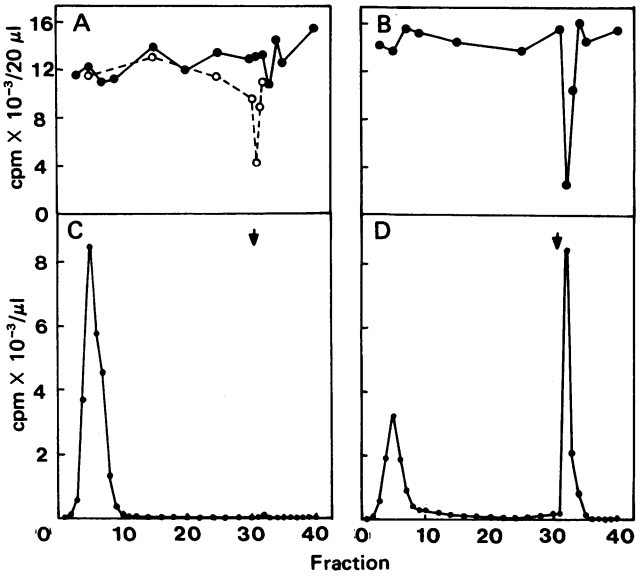

FIG. 2. Purification of the inhibitor by DEAE-cellulose chromatography. Columns of poly(I)·poly(C)-agarose (150 µl) that had received 100 µl of the postribosomal supernatants from mock- or interferon-treated cells were washed extensively and then incubated at 30° for 11 hr in the presence of [^{3}H]ATP (100 µl; 1 mM; 0.25 mCi/ml). [^{3}H]ATP (Schwarz/Mann) had been purified by binding to a DEAE-cellulose column at 20 mM KCl and elution at 50 mM KCl. The eluates (1 ml) from the poly(I)·poly(C)-agarose columns were collected and applied to columns (0.3 × 2 cm) of DEAE-cellulose equilibrated with 10 mM Hepes, pH 7.6/50 mM KCl/1.5 mM Mg acetate/20% glycerol. The columns were washed with this buffer and 30 fractions (0.45 ml) were collected before the KCl concentration was increased to 0.3 M (indicated by the arrows). No more radioactivity eluted upon a further step to 1.0 M KCl. Fractions were assayed for their content of ^{3}H-labeled material (*C* and *D*) and for their ability to inhibit cell-free protein synthesis in extracts of untreated chick cells directed by VSV mRNA (*A* and *B*) after 1:250 (---○---) or 1:125,000 (—●—) dilution. Endogenous protein synthesis amounted to 3500 cpm/20 µl and has been subtracted from the results shown. (*A* and *C*) Reaction products from the enzyme preparation derived from mock-interferon-treated cells. (*B* and *D*) Products from the enzyme from interferon-treated cells.

the column-bound state, the enzyme that generated the inhibitor was stable for weeks at 4° and could be incubated repeatedly at 30°. No attempts have yet been made to elute the enzyme from the column.

The eluates from columns that had been incubated overnight with [^{3}H]ATP were applied to DEAE-cellulose columns equilibrated with buffer containing 50 mM KCl. In the interferon case, all inhibitory activity and a large fraction of the isotopic label were eluted from the column only after a step to 300 mM KCl (Fig. 2 *B* and *D*). In the mock interferon case, the first peak contained almost all the label, in the form of unreacted ATP and ADP. However, a small peak of label, amounting to 0.4% of the interferon peak, eluted at 300 mM KCl, and trace amounts of inhibitory activity were also detected in these fractions (Fig. 2 *A* and *C*).

The ^{3}H-labeled inhibitor, freed from residual ATP by DEAE-cellulose chromatography, was compared with the low molecular weight inhibitor synthesized in a similar manner by an enzyme from interferon-treated mouse cells (17, 18). (The purified ^{3}H-labeled mouse inhibitor was generously supplied by Ian Kerr.) Fig. 3 shows the results of thin-layer chromatography on polyethyleneimine (PEI)-cellulose plates of the two inhibitors and the products of their digestion with bacterial alkaline phosphatase [orthophosphoric monoester phos-

Table 1. dsRNA-dependent formation of an inhibitor of protein synthesis

Post-ribosomal supernatant	Preincubation conditions		Dilution into assay	% of control protein synthesis
	Poly(I)·poly(C)	ATP		
—	−	+	0.05	100 (control)
—	+	+	0.05	100
Mock	−	+	0.05	94
Mock	+	+	0.05	99
Interferon	−	+	0.05	96
Interferon	+	+	0.05	7
Interferon	+	+	0.02	11
Interferon	+	+	0.01	19
Interferon	+	+	0.005	36
Interferon	+	+	0.001	78
Interferon	+	+	0.05	7
Interferon	+	+	0.01	36
Interferon	+	+	0.05	89
Interferon	+	$+\beta\gamma(CH_2)$ATP	0.05	88
Interferon	+	$+\beta\gamma(CH_2)$ATP	0.01	97
Interferon	+poly(rI)·poly(dC)‡	+ATP	0.05	92

Postribosomal supernatants from mock- or interferon-treated chick embryo cells were preincubated with or without 1 μg of poly(I)·poly(C) per ml and in the presence of 1 mM ATP or β,γ-methylene-ATP (P-L Biochemicals). After 30 min at 30°, the preincubation mixtures were diluted and assayed for their ability to inhibit protein synthesis catalyzed by extracts of untreated chick cells, programmed with VSV mRNA. These extracts were insensitive to inhibition by dsRNA itself (20). Endogenous protein synthesis was about 17% of the VSV mRNA-stimulated value and has been subtracted. The results shown are averages derived from several separate experiments.

* After 30 min at 30°, these preincubation mixtures were heated at 90° for 10 min and centrifuged at 10,000 × *g* for 10 min, and the supernatants were assayed for inhibitory activity.

† This preincubation mixture was heated at 90° for 10 min before being preincubated at 30° for 30 min. It was then centrifuged at 10,000 × *g* for 10 min, and the supernatant was assayed for inhibitory activity.

‡ At 1 μg/ml.

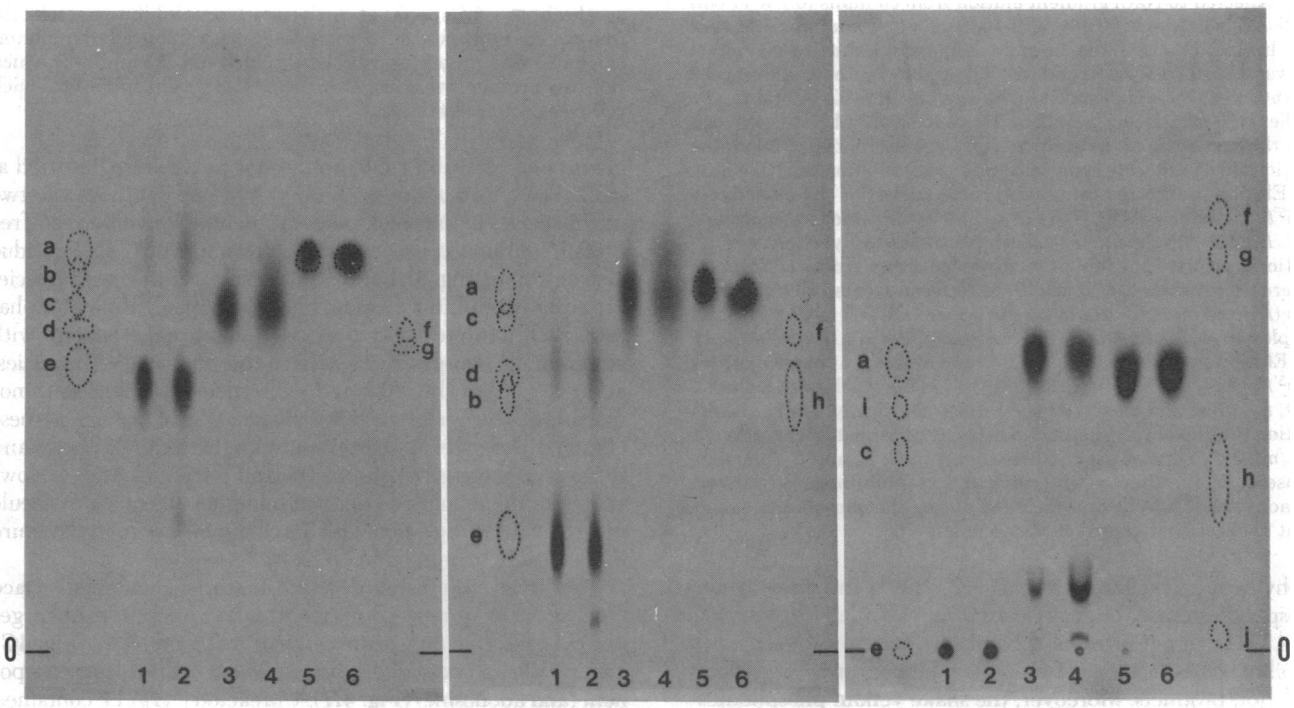

FIG. 3. Thin-layer chromatography of the inhibitory oligonucleotides synthesized by the enzymes derived from interferon-treated mouse and chick cells. The inhibitory oligonucleotides, labeled with [^{3}H]ATP, were purified on DEAE-cellulose and subjected to thin-layer chromatography on PEI-cellulose plates before and after enzymic digestion. Chromatography solvents used were: (*Left*) 0.75 M KH_2PO_4, pH 3.4 (22); (*Center*) 1.0 M LiCl; (*Right*) 1.0 M acetic acid. Lanes: 1 and 2, mouse and chick inhibitors, respectively, before digestion; 3 and 4, mouse and chick inhibitors after digestion with bacterial alkaline phosphatase (Sigma; 1 unit/ml; 37°; 1 hr); 5 and 6, mouse and chick inhibitors after digestion with snake venom phosphodiesterase (Worthington; 1 unit/ml; 37°; 1 hr). Unlabeled 5′-AMP was included as a marker in lanes 5 and 6, and its position, visualized under UV light, is indicated. Other markers were run in separate lanes and visualized similarly but have been superimposed in this figure. They were: a, 5′-AMP; b, 5′-ADP; c, 3′-AMP; d, adenosine; e, 5′-ATP; f, $A^{2'}p^{5'}A$; g, $A^{3'}p^{5'}A$; h, $(Ap)_2A$ (3′→5′); i, 2′-AMP; j, $(Ap)_3A$ (3′ → 5′). ^{3}H-labeled material was visualized by fluorography on presensitized x-ray film (23) after the thin-layer plates had been impregnated with 20% 2,5-diphenyloxazole in acetone. The origin (0) is at the bottom.

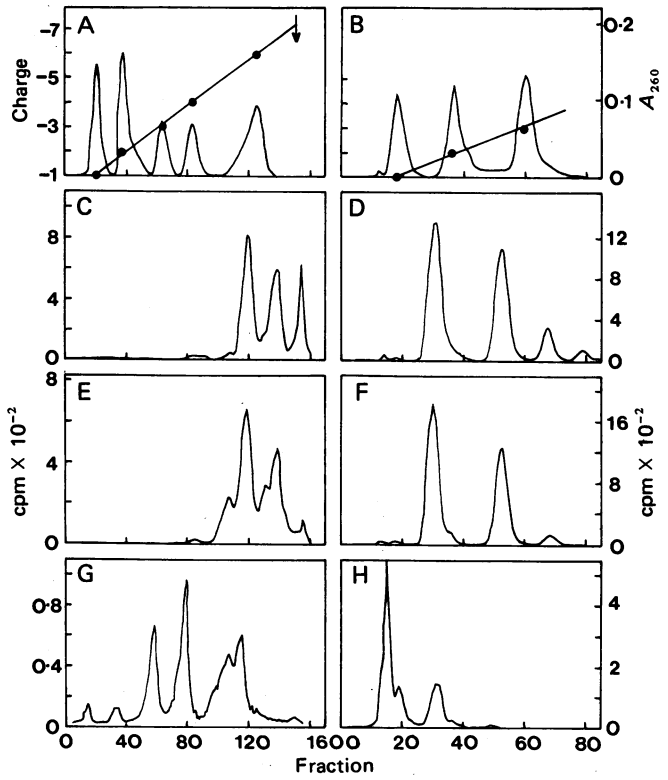

FIG. 4. Chromatography on columns of DEAE-cellulose eluted with gradients of NaCl containing 7 M urea. The inhibitory oligonucleotides, labeled with [^{3}H]ATP, were purified on DEAE-cellulose and subjected to NaCl gradient elution from columns (0.7 × 17 cm) of DEAE-cellulose in 50 mM Tris·HCl, pH 8.0, containing 7 M urea (24). In *A, C, E,* and *G*, the linear gradient (80 ml, starting in fraction 21) was 0.05–0.15 M NaCl and was followed by buffer containing 0.3 M NaCl at the position shown by the arrow. In *B, D, F,* and *H*, the gradient (80 ml, starting in fraction 21) was 0.05–0.3 M NaCl, although only the first 40 ml of the gradient is shown. Aliquots (0.6 ml) of the fractions (0.65 ml) were assayed by liquid scintillation counting (26). (*A*) Elution profile and calibration curve of the net charge markers $A^{2'}p^{5'}A$, 5′-AMP, 5′-ADP, 5′-ATP, and $pp^{5'}G^{3'}pp$ which were included in *C, E,* and *G* and detected spectrophotometrically. (*C, E,* and *G*) Elution profiles of the inhibitors made by enzymes derived from interferon-treated mouse L cells (*C*), interferon-treated chick embryo cells (*E*), and mock interferon-treated chick cells (*G*) (a 40-fold larger sample was loaded than in *E*, and residual ATP and ADP are evident). (*B*) Elution profile and calibration curve of the net charge markers $A^{2'}p^{5'}A$, $(Ap)_2A(3' \rightarrow 5')$, and $(Ap)_3A$ $(3' \rightarrow 5')$ which were included in *D, F,* and *H* and detected spectrophotometrically. (*D, F,* and *H*) Elution profiles of the products of alkaline phosphatase digestion (1 unit/ml; 37°; 1 hr) of the samples shown in *C, E,* and *G*—namely, mouse, chick (interferon), and chick (mock) inhibitors, respectively. In each case, [^{14}C]ATP was included during the phosphatase treatment to check that digestion was complete.

phohydrolase (alkaline optimum), EC 3.1.3.1] and snake venom phosphodiesterase (oligonucleate 5′-nucleotidehydrolase, EC 3.1.4.1). In three different solvent systems, no differences in mobility were detected between the inhibitors or between their digestion products. Moreover, the snake venom phosphodiesterase products comigrated with 5′-AMP. Both inhibitor preparations showed evidence of more than one labeled component, and the products of alkaline phosphatase digestion were clearly resolved into three species by chromatography in 1.0 M acetic acid (Fig. 3 *right*).

Further evidence for multiple components in both inhibitor preparations came from their profiles on DEAE-cellulose columns developed with NaCl gradients containing 7 M urea (24). In both preparations, the major species had apparent net

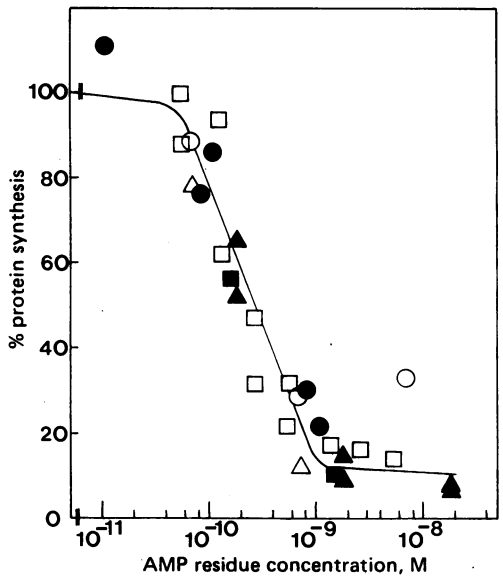

FIG. 5. Assay of the inhibitors made by the mouse and chick cell-derived enzymes for their ability to inhibit protein synthesis in cell-free extracts of mouse and chick cells. Protein synthesis was assayed in extracts of mouse L cells programmed with encephalomyocarditis virus RNA (10) and in extracts of primary chick embryo cells programmed with VSV mRNA. Endogenous incorporation has been subtracted. The concentrations of the inhibitor preparations in terms of their content of AMP residues were calculated from the specific isotopic activity of the [^{3}H]ATP used for synthesis. In the case of the chick inhibitor, the calculated concentration was confirmed by A_{260} measurements. ▲, Chick (interferon) inhibitor, chick cell-free protein synthesis; ○, chick (interferon) inhibitor, mouse cell-free protein synthesis; ■, −5.7 species of chick (interferon) inhibitor, chick cell-free protein synthesis; △, −6.6 species of chick (interferon) inhibitor, chick cell-free protein synthesis; ●, chick (mock) inhibitor, chick cell-free protein synthesis; □, mouse (interferon) inhibitor, chick cell-free protein synthesis.

charges of −5.7 and −6.6, and minor peaks were detected at −5.2, −6.1, and about −7.3 (Fig. 4 *C* and *E*). Both the two major species in the chick preparation inhibited chick cell-free protein synthesis at concentrations down to 1 nM (AMP residue concentration, Fig. 5), but it is not yet clear if the minor species are equally inhibitory. Upon digestion with alkaline phosphatase, both inhibitor preparations yielded major species with apparent net charges of −1.7 (from the −5.2 and −5.7 species) and −2.7 (from the −6.1 and −6.6 species) as well as minor species with higher net negative charges (Fig. 4 *D* and *F*). These charge differences are consistent with the removal of two and three phosphate groups from tri- and tetranucleotides. However, like the mouse-cell derived inhibitor, the chick molecule was resistant to digestion with T2 ribonuclease, so its structure is not simply $(p)ppA^{3'}p^{5'}A^{3'}p^{5'}A^{3'}(p^{5'}A)_{OH}$.

The mock-interferon-derived material contained trace amounts of inhibitory components with apparent net charges of −5.1 and −5.6 (and some residual ATP and ADP) (Fig. 4*G*) and, on phosphatase digestion, yielded a −1.6 charge component (and adenosine) (Fig. 4*H*). Unreacted [^{3}H]ATP contained only ATP, ADP, and AMP peaks (not shown).

The inhibitors made by the mouse cell- and the chick cell-derived enzymes were equally efficient in blocking protein synthesis in cell-free extracts of untreated mouse L cells and primary chick embryo cells, and they gave 50% inhibition at concentrations equivalent to an AMP concentration of about 0.3 nM (Fig. 5). In addition, the specific inhibitory activity of the preparation derived from mock-interferon-treated chick cells (calculated on the basis of its content of −5.1 and −5.6

charge species) was the same as that of the preparation derived from interferon-treated cells. Its concentration was 0.2% of the interferon-derived preparation.

Above 1 nM (AMP residue concentration), both oligonucleotide preparations inhibited cell-free protein synthesis by about 95% (Fig. 5). This inhibition was not specific for mRNAs of viral origin because the translation of poly(A)-containing RNA from uninfected mouse L cells was equally sensitive to inhibition. However, the translation of poly(U) was less sensitive, being inhibited by only 20–30% at inhibitor concentrations up to 1 μM (AMP residue concentration). Protein synthesis directed by brome mosaic virus RNA in a wheat germ cell-free system was insensitive to inhibition by the oligonucleotide (data not shown).

DISCUSSION

Using procedures developed by Kerr and his coworkers (17, 18) we have isolated from interferon-treated chick cells an enzyme that synthesizes an oligonucleotide(s) of unusual structure that is capable of inhibiting cell-free protein synthesis at subnanomolar concentrations. Like the enzyme extracted from mouse L cells, the chick cell-derived enzyme is activated by dsRNA and uses ATP as a substrate. The enzyme can be handled most conveniently when bound to a column of poly(I)·poly(C)-agarose, and under these conditions it will synthesize the inhibitory oligonucleotide upon perfusion of the column with ATP. The structure of the chick inhibitor has yet to be determined, but the results reported here indicate that it is probably identical to the interferon-treated mouse cell-derived inhibitor (17, 18), whose structure has recently been shown to be $ppp5'A2'p5'A2'p5'A_{OH}$ (25). The fact that the mouse and chick cell-derived inhibitors were functionally interchangeable in inhibiting protein synthesis in mouse and chick cell-free systems supports the idea that they are identical and indicates that the species-specificity of interferon action does not extend to the subcellular level. Moreover, the discovery of such similar responses to interferon treatment shown by cells from such disparate origins as mouse L cells and primary chick embryo cells supports the idea that the inhibitory oligonucleotide is involved in the mechanism of action of interferon. Its lack of discrimination between host and viral mRNA is a puzzling aspect of the results, which may reflect either a shortcoming of the cell-free system assay or our incomplete understanding of the effects of interferon treatment in intact cells.

Trace amounts of the inhibitor were synthesized by the enzyme preparation derived from mock-interferon-treated primary chick embryo cells. The possibility that these cells had been exposed to minute doses of endogenous interferon, either in the embryo or in tissue culture, has not been eliminated. However, the result raises the possibility that the enzyme responsible for synthesis of the inhibitor is a constituent of some normal cells and is vastly increased upon interferon treatment. In this respect, it is interesting that the same enzyme activity has been identified in rabbit reticulocyte lysates (27) and in extracts of mouse 3T6 cells (C. Colby, A. Jarvis, C. N. White, and L. A. Ball, unpublished data). Perhaps the inhibitory oligonucleotide is involved in the regulation of protein synthesis in normal cells and is thus of significance beyond the interferon system.

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1. Ho, M. & Armstrong, J. A. (1975) *Annu. Rev. Microbiol.* **29,** 131–161.
2. Taylor, J. (1964) *Biochem. Biophys. Res. Commun.* **14,** 447–451.
3. Friedman, R. M. & Sonnabend, J. A. (1964) *Nature* **203,** 366–367.
4. Friedman, R. M., Metz, D. H., Esteban, R. M., Tovell, D. R., Ball, L. A. & Kerr, I. M. (1972) *J. Virol.* **10,** 1184–1198.
5. Falcoff, E., Falcoff, R., Lebleu, B. & Revel, M. (1973) *J. Virol.* **12,** 421–430.
6. Gupta, S. L., Sopori, M. L. & Lengyel, P. (1973) *Biochem. Biophys. Res. Commun.* **54,** 777–783.
7. Samuel, C. E. & Joklik, W. K. (1974) *Virology* **58,** 476–491.
8. Kerr, I. M., Brown, R. E. & Ball, L. A. (1974) *Nature* **250,** 57–59.
9. Content, J., Lebleu, B., Nudel, U., Zilberstein, A., Berissi, H. & Revel, M. (1975) *Eur. J. Biochem.* **54,** 1–10.
10. Kerr, I. M., Brown, R. E., Clemens, M. J. & Gilbert, C. S. (1976) *Eur. J. Biochem.* **69,** 551–561.
11. Roberts, W. K., Clemens, M. J. & Kerr, I. M. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 3136–3140.
12. Roberts, W. K., Hovanessian, A. G., Brown, R. E., Clemens, M. J. & Kerr, I. M. (1976) *Nature* **264,** 477–480.
13. Lebleu, B., Sen, G. C., Shaila, S., Cabrer, B. & Lengyel, P. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 3107–3111.
14. Zilberstein, A., Federman, P., Shulman, L. & Revel, M. (1976) *FEBS Lett.* **68,** 119–124.
15. Brown, G. E., Lebleu, B., Kawakita, M., Shaila, S., Sen, G. C. & Lengyel, P. (1976) *Biochem. Biophys. Res. Commun.* **69,** 114–122.
16. Sen, G. C., Lebleu, B., Brown, G. E., Kawakita, M., Slattery, E. & Lengyel, P. (1976) *Nature* **264,** 370–373.
17. Hovanessian, A. G., Brown, R. E. & Kerr, I. M. (1977) *Nature* **268,** 537–540.
18. Kerr, I. M., Brown, R. E. & Hovanessian, A. G. (1977) *Nature* **268,** 540–542.
19. Ball, L. A. & White, C. N. (1978) *Virology* **84,** in press.
20. Ball, L. A. & White, C. N. (1978) *Virology* **84,** in press.
21. Ball, L. A. & White, C. N. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 442–446.
22. Cashel, M., Lazzarini, R. A. & Kalbacher, B. (1969) *J. Chromatogr.* **40,** 103–109.
23. Laskey, R. A. & Mills, A. D. (1975) *Eur. J. Biochem.* **56,** 335–341.
24. Banerjee, A. K. & Shatkin, A. J. (1971) *J. Mol. Biol.* **61,** 643–653.
25. Kerr, I. M. & Brown, R. E. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 256–260.
26. Rhodes, D. P., Moyer, S. A. & Banerjee, A. K. (1974) *Cell* **3,** 327–333.
27. Hovanessian, A. G. & Kerr, I. M. (1978) *Eur. J. Biochem.*, in press.