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Specific antiviral activity of a poly(L-lysine)-conjugated oligodeoxyribonucleotide sequence complementary to vesicular stomatitis virus N protein mRNA initiation site

(antisense oligonucleotides/intracellular delivery/virus inhibition)

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ABSTRACT **Antisense oligonucleotides represent an interesting tool for selective inhibition of gene expression, but their efficient introduction within intact cells proved to be difficult to realize. As a step toward this goal, small (13- or 15-mer) synthetic oligodeoxyribonucleotides have been coupled at their 3′ ends to ε-amino groups of lysine residues of poly(L-lysine) (M_r, 14,000). A 15-mer oligonucleotide-poly(L-lysine) conjugate complementary to the initiation region of vesicular stomatitis virus (VSV) N-protein mRNA specifically inhibits the synthesis of VSV proteins and exerts an antiviral activity against VSV when added in the cell culture medium at doses as low as 100 nM. Neither synthesis of cellular proteins nor multiplication of encephalomyocarditis virus was affected significantly by this oligonucleotide conjugate. The data suggest that oligonucleotide-poly(L-lysine) conjugates might become effective for studies on gene expression regulation and for antiviral chemotherapy.**

Naturally occurring RNA sequences that negatively control the expression of certain genes have been discovered recently in prokaryotes (1). Their inhibitory action on gene expression probably arises from specific hybridization of short transcripts to complementary sequences in mRNAs (2, 3). No such sequences have yet been documented in eukaryotes.

Several lines of evidence suggest that selective inhibition of gene expression can be achieved artificially through the introduction and expression of oligonucleotidic interfering sequences in intact cells. RNA and DNA oligonucleotide sequences effectively block translation in cell-free experiments (4–6). An early study reported the antiviral activity of a 13-mer oligodeoxyribonucleotide in intact Rous sarcoma virus-infected fibroblasts (7). Works by Inouye and his colleagues (8, 9) on prokaryotes and by Izant and Weintraub (3, 10) on eukaryotes demonstrated that exogenous antisense sequences could be used for specific regulation of gene expression in intact cells. Antisense RNAs were generated by transcription of sequences inserted in reverse orientation downstream from various promoters on recombinant plasmids introduced into intact cells by standard transfection techniques. These initial observations were followed by a flurry of works (see refs. 1 and 11 for reviews) aimed at probing the possibility of regulating gene expression through antisense RNAs in various biological systems, at deriving tools to achieve their efficient introduction and expression in cultured cells or in multicellular organisms, and at studying mechanism(s) involved in such controls. This technology indeed provides an alternative to standard mutational analysis, which is often difficult and sometimes impossible to achieve in diploid cells or organisms. Alternatively, it might become a pharmacological tool for the negative regulation of expression of deleterious cellular (e.g., deregulated oncogenes) or foreign (e.g., viral) genes.

As briefly mentioned above, antisense sequences were transiently expressed from recombinant plasmids introduced in intact cells by standard transfection studies (e.g., see refs. 8 and 10) or by microinjection (e.g., see refs. 3, 12, and 13). In a few cases, cells were engineered to express their own antisense RNA from stably integrated chromosomal genes (14–16). Using a rather different approach, Miller, Ts'o, and their colleagues synthesized nonionic methylphosphonate oligodeoxyribonucleotide analogues, which possess increased resistance to nucleolytic degradation and penetrate the plasma membrane of mammalian cells growing in culture while retaining their ability to hybridize specifically to complementary RNA or DNA sequences (see refs. 17 and 18 for reviews). Short methylphosphonate oligodeoxyribonucleotides (8 or 9 nucleotides long) complementary to the initiation codon region of NS protein of vesicular stomatitis virus (VSV) (18) or to the acceptor splice junction of an immediate-early herpes simplex virus type 1 (HSV-1) pre-mRNA (19) reduced virus titers by ≈1 logarithmic unit when infected cells were treated with 150 μM and 75 μM oligomers, respectively. Similarly, C. Hélène and co-workers used acridine-conjugated oligodeoxyribonucleotides to regulate the expression of several viral genes in cell-free extracts (6, 20) and in intact cells (C. Hélène, personal communication).

We have derived an alternative method for the delivery of antisense sequences to intact cells. It consists of chemical conjugation of oligodeoxyribonucleotides to poly(L-lysine) ε-amino groups through a *N*-morpholine ring (21). Poly(L-lysine) (M_r, 14,000) conjugated with a 15-nucleotide-long sequence complementary to the initiation codon of VSV N protein was shown to specifically inhibit VSV protein synthesis and VSV multiplication when incubated with cells at a concentration as low as 100 nM in the culture medium.

MATERIALS AND METHODS

Chemicals. Media and fetal calf serum for cell culture were obtained from Eurobio (Paris). Poly(L-lysine) (M_r, 14,000), bacterial alkaline phosphatase (type III-R), myokinase (type V), spermine, and sodium cyanoborohydride were purchased from Sigma. T4 RNA ligase (RNase- and DNase-free) and unlabeled pCp were purchased from Pharmacia and creatine kinase was from Boehringer Mannheim. 5′ [^{32}P]pCp (111 TBq/mmol) and [^{35}S]methionine (22–30 TBq/mmol) were from Amersham and ^{14}C-labeled molecular weight protein standards were from New England Nuclear.

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Abbreviations: VSV, vesicular stomatitis virus; EMCV, encephalomyocarditis virus; moi, multiplicity of infection.

Cells and Viruses. L929 cells were grown in minimum essential medium (MEM) supplemented with 5% (vol/vol) fetal calf serum, bactotryptose phosphate broth (3 g/liter), glucose (3.4 g/liter), penicillin (60 units/ml), and streptomycin (50 mg/ml). The Indiana strain of VSV or encephalomyocarditis virus (EMCV) was grown in L929 cell monolayers and titrated by an end-point method (22).

Synthesis of Oligonucleotides. Oligodeoxyribonucleotides were synthesized using a Biosearch Sam One DNA synthesizer using the phosphotriester method. Deprotected oligonucleotides were purified on a Sephadex G-50 column equilibrated with 10 mM triethylamine bicarbonate buffer (pH 7.5) (TEAB). TEAB was eliminated by evaporation and the purity of this fraction was assayed by PAGE (23). If necessary, the oligonucleotides were further purified by preparative PAGE in conditions similar to those of analysis.

Addition of pCp to Oligodeoxyribonucleotides. We adapted conditions from published reports (24, 25). The oligodeoxyribonucleotide (80 nmol) was lyophilized with 320 nmol of pCp, 1.85 MBq of 5′ [^{32}P]pCp, 80 nmol of spermine, 1.8 μmol of creatine phosphate, and 4.5 nmol of ATP. The residue was redissolved in 30 μl of ligation buffer (50 mM Hepes, pH 7.9/10 mM $MnCl_2$/20 mM dithiothreitol). Creatine kinase and myokinase (1.7 units of each) in 5 μl of ligation buffer/30% (vol/vol) glycerol were added, and the reaction was initiated by the addition of T4 RNA ligase (10–15 μl, to a final concentration of 23 μM). The reaction mixture was incubated at 18°C for 6 days. Unbound pCp and salts were removed by chromatography on a Biogel P6-DG column equilibrated and developed with 20 mM TEAB.

We measured radioactivity and A_{260} of each fraction. Fractions containing the oligodeoxyribonucleotide pCp were pooled and evaporated to dryness.

Conjugation of Oligodeoxyribonucleotide pCp to Poly(L-Lysine). Dried oligodeoxyribonucleotide pCp was solubilized in 50 μl of 10 mM TEAB and incubated for 1 hr at 37°C with 1 unit of bacterial alkaline phosphatase. The dephosphorylated oligodeoxyribonucleotide was purified on Biogel P6-DG in 10 mM TEAB and lyophilized. The residue was solubilized in 100 μl of sodium metaperiodate (1 μmol in 0.1 M sodium acetate buffer, pH 4.75), and the reaction mixture was incubated for 2 hr at 0°C–4°C in the dark. Poly(L-lysine) (100 μl) (80 nmol in 0.2 M phosphate buffer, pH 8.0) and 100 μl of sodium cyanoborohydride (10 μmol in 0.2 M phosphate buffer, pH 8.0) were then added and incubation was allowed to proceed for 2 hr at room temperature. The sample was then loaded on a Sephadex G-50 column equilibrated and eluted with 0.1 M ammonium acetate buffer (pH 4.5). Each fraction was assayed for oligodeoxyribonucleotide-poly(L-lysine) content using Lowry's method (26), by absorbance at 260 nm, and by measurement of radioactivity.

Protein Synthesis Assay. L929 cell cultures (3–5 × 10^5 cells) were washed twice with methionine-free minimal essential medium (MEM) and labeled for 45 min at 37°C with 185 kBq of [^{35}S]methionine in 500 μl of methionine-free MEM. Proteins were extracted and analyzed by gradient $NaDodSO_4$/PAGE (7.5–15%, wt/vol) as described (21). Protein distribution in the gels was analyzed by fluorography (27) and quantitated with a Vernon densitometer on the autoradiographs.

RESULTS

Conjugation of Oligodeoxyribonucleotides and Poly(L-Lysine). Oligoribonucleotides can be covalently linked to poly(L-lysine) via a *N*-morpholine (azahexopyranose) ring after periodate oxidation of their 3′-terminal ribose residue (28, 29). We had to add an oxidizable 3′-terminal ribose to use this method with synthetic oligodeoxyribonucleotides. This operation can be performed with T4 RNA ligase (24, 25). We were able to ligate [^{32}P]pCp and oligodeoxyribonucleotides with yields of >85%.

Oxidation of the 3′-terminal cytidylic residue and its coupling to ε-amino groups of poly(L-lysine) were then performed as described for the conjugation of (2′–5′)$(A)_n$ to poly(L-lysine) (21). An outline of the overall procedure used for the conjugation of oligodeoxyribonucleotides to poly(L-lysine) is given in Fig. 1. The length of the oligodeoxyribonucleotide was defined by taking into account published reports (1, 13, 18) and our previous experience in handling (2′–5′)$(A)_n$-poly(L-lysine) conjugates. Two to five (2′–5′)(A) tetramers could be coupled to one molecule of poly(L-lysine) of 66 amino acid residues (M_r, 14,000) without any precipitation of the conjugate and delivered in a functional form within intact cells. On the other hand, a minimal length was necessary to hybridize with the target mRNA, and longer oligonucleotidic sequences have a higher probability of specific hybridization. The molecular weight of the poly(L-lysine) carrier also seems of importance for efficiency (data not shown). The best results were obtained by coupling oligomers ranging in size from 10 to 15 nucleotides to M_r 14,000 poly(L-lysine).

The reasoning for identification of a possible target for antisense oligodeoxyribonucleotide in this system was as follows. The VSV genome consists of a single RNA negative strand of ≈11,000 nucleotides, encoding five proteins: N, NS, M, G, and L (30, 31). The N protein coats the entire length of the virion RNA and plays roles in the replication of the genome and possibly in transcription (32). In most studies so far, the best inhibitory effect of synthetic anti-mRNA oligonucleotides was obtained with oligomers complementary to the 5′ region of the target mRNA, including or not the AUG initiation codon (e.g., see refs. 9, 10, and 18).

Two oligodeoxyribonucleotides were thus synthesized. The first (oligodeoxyribonucleotide 1 in Fig. 2) was 15 nucleotides long and complementary to the 5′ end region of the mRNA coding for the N protein of VSV. It may hybridize with the major ribosome protected initiation site around the 5′ proximal AUG codon. The second (oligodeoxyribonucleotide 2 in Fig. 2) was an internal sequence of 13 nucleotides located in the middle of the coding region of this 1326-nucleotide mRNA (33). These synthetic oligomers were covalently linked to poly(L-lysine) as described above with an average molar ratio of 0.5 and a 25–30% yield.

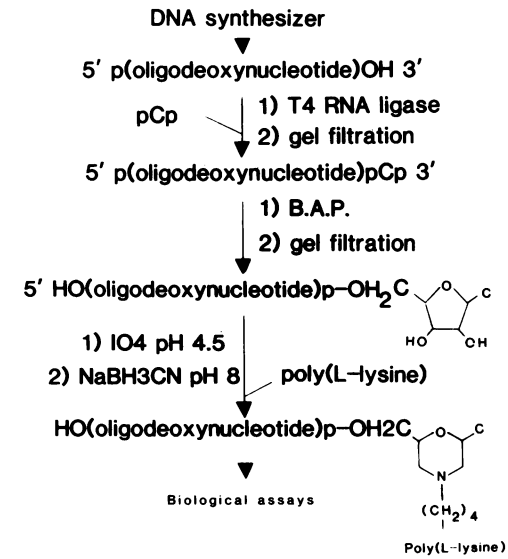

FIG. 1. Preparation of oligodeoxyribonucleotide-poly(L-lysine) conjugates.

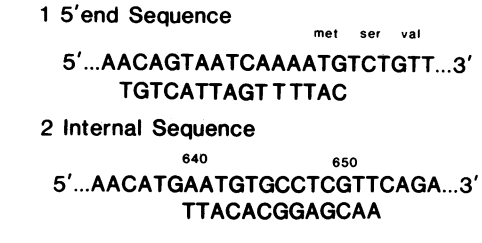

FIG. 2. Partial nucleotide sequence of the VSV mRNA encoding the N protein and location of the complementary synthetic oligodeoxyribonucleotides; the sequences of the synthetic oligomers coupled to poly(L-lysine) are shown below the mRNA. 1: Oligodeoxyribonucleotide complementary to the translation initiation site. 2: Oligodeoxyribonucleotide complementary to an internal sequence in the middle of the coding region.

A search with the Citi 2 GeneBank program did not reveal any homology between our synthetic oligodeoxyribonucleotides and other sequenced genes.

Antiviral Effect of Oligodeoxyribonucleotide-Poly(L-Lysine) Conjugates. Conjugates complementary to the 5′ end sequence and defined as above were incubated with L929 cells 2 hr prior to infection with VSV at a multiplicity of infection (moi) of 1. Virus titers were determined 15 hr later by end-point assay (22). Preliminary experiments indicate that the conjugate is still efficient when added shortly (e.g., 2 hr) after VSV infection (data not shown).

As reported in Table 1 for three independent experiments, a dose-dependent reduction of VSV yield was observed in every case. The antiviral activity was detectable with conjugate concentration in the culture medium as low as 100 nM and reached >95% inhibition at 400 nM. Furthermore, and whatever the concentration of the conjugate, no significant effect on EMCV production was observed, unlike antiviral activity on VSV. This demonstrated the specificity of the antiviral action of the 5′ end sequence conjugated to poly(L-lysine).

In addition, no significant antiviral activity was observed when L929 cells were incubated with a mixture of poly(L-lysine) and 5′ end sequence oligomers. In agreement with our previous data on (2′–5′)$(A)_n$ oligonucleotides (21), this result demonstrates that a covalent linkage of the oligodeoxyribonucleotide to poly(L-lysine) is necessary to obtain a biological activity. Moreover, no effect on virus multiplication was observed when cells were incubated with poly(L-lysine) (M_r, 14,000) at doses up to 2 μM.

Finally, the incubation of cells with a conjugate associating poly(L-lysine) to an oligodeoxyribonucleotide complementary to an internal sequence of VSV mRNA coding for N protein had no significant effect on VSV titer as measured 15 hr after infection (Table 1). The location of the oligomer hybridization site thus influences the efficiency of inhibition by the conjugate in this system. This is in agreement with a number of published data (3, 10, 18) and provides further support for the hypothesis that 5′ regions of the mRNA are better targets for translation inhibition by antisense oligonucleotides.

Effect of Oligodeoxyribonucleotide-Poly(L-Lysine) Conjugates on Viral and Cellular Protein Synthesis. As a first approach to the mechanism of the antiviral activity of these antisense poly(L-lysine) conjugates, their effects on protein synthesis in uninfected and in VSV-infected cells were examined.

L929 cells were incubated with increasing concentrations of the 5′ end sequence conjugated or not to poly(L-lysine) 2 hr before infection with VSV at a moi of 1. Cells were pulse-labeled with [^{35}S]methionine 5 hr after infection and acid-insoluble polypeptides were analyzed by NaDodSO$_4$/PAGE electrophoresis as shown in Fig. 3. The 5′ end sequence oligodeoxyribonucleotide conjugate led to a dose-dependent reduction (lanes 2–5) in the synthesis of VSV-coded N protein. It is interesting to note that the synthesis of VSV-coded M protein was reduced in the same way, while the synthesis of cellular proteins was increased concomitantly. When cells were incubated with a mixture of 200 pmol of the 5′ end sequence oligodeoxyribonucleotide and 400 pmol of poly(L-lysine) (lane 7)—i.e., in the same molar ratio and at the same concentration as in lane 5—no inhibitory effect on viral protein synthesis was observable as compared with the control (lane 2). This confirms the necessity of a covalent linkage between the oligodeoxyribonucleotide sequence and the polypeptide carrier for its biological activity.

In a separate experiment, protein synthesis was measured in the absence (lane 8) or in the presence (lane 6) of 200 pmol of 5′ end sequence linked to poly(L-lysine). The conjugate clearly had no effect on cellular protein synthesis in uninfected cells and the increase of cellular protein synthesis observed in VSV-infected cells probably results from a partial reversal of VSV-induced shut-off of host protein synthesis.

Densitometric scanning of lanes 2–5 was carried out to quantify the effect of 5′ end sequence conjugate on viral protein synthesis. The amount of viral protein N or M was normalized to a M_r 55,000 cellular protein peak (shown by arrow in Fig. 3). As illustrated in Fig. 4, this ratio decreases from 25 to 0.1 for N protein and from 13 to a nonmeasurable level for M protein in parallel with the increase of the oligodeoxyribonucleotide concentration.

As illustrated in Fig. 5, total acid-precipitable radioactivity remained unchanged when uninfected cells were incubated with increasing concentrations of the 5′ end sequence conjugate. A dose-dependent increase in protein synthesis was observed when cells were treated with increasing concentrations of the same 5′ end sequence conjugate, challenged with VSV (moi = 1) 2 hr later and pulse-labeled with [^{35}S]methionine 6 hr after infection. Once again, a mixture of unconjugated 200-pmol oligodeoxyribonucleotide and 400-pmol

Table 1. Antiviral activity of oligonucleotide-poly(L-lysine) conjugates

Oligonucleotide concentration, nM	5′ end oligonucleotide sequence linked to poly(L-lysine)				VSV titer	
	VSV titer				Mixture of 5′ end sequence and poly(L-lysine)	Internal oligonucleotide sequence linked to poly(L-lysine)
	Exp. 1	Exp. 2	Exp. 3	EMCV titer		
0	4.2×10^8	5.8×10^8	1.0×10^8	5.8×10^7	3.1×10^8	1.0×10^8
100	1.7×10^8 (40%)	ND	3.1×10^7 (30%)	3.1×10^7 (53%)	2.3×10^8 (75%)	7.8×10^7 (78%)
200	7.8×10^6 (2%)	2.3×10^7 (4%)	1.7×10^7 (15%)	3.1×10^7 (53%)	4.2×10^8 (135%)	7.8×10^7 (78%)
400	3.0×10^6 (0.7%)	1.7×10^6 (0.4%)	4.2×10^6 (4%)	5.8×10^7 (100%)	2.3×10^8 (75%)	7.8×10^7 (78%)

L929 cells were infected with VSV or EMCV, at moi = 1, 2 hr after incubation with the conjugated or unconjugated oligodeoxyribonucleotide. Virus titers determined by end-point assay 15 hr later are expressed in plaque-forming units/ml or as a percentage of control value. ND, not done.

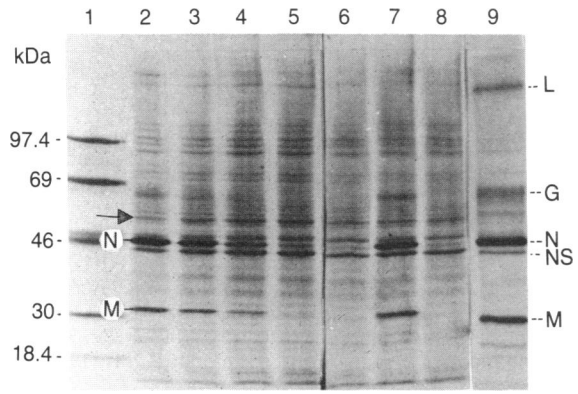

FIG. 3. NaDodSO$_4$/PAGE analysis of the inhibition of viral protein synthesis by mRNA complementary oligodeoxyribonucleotide-poly(L-lysine) conjugate. L929 cells were treated with no (lane 2), 100 nmol (lane 3), 200 nmol (lane 4), or 400 nmol (lane 5) of the 5′ end sequence poly(L-lysine) conjugate in 500 μl of medium, infected with VSV [multiplicity of infection (moi) = 1] and pulse-labeled with [^{35}S]methionine, 5 hr after infection. As control, cells were treated with 400 nmol of 5′ end sequence poly(L-lysine) conjugate and left uninfected (lane 6), or treated with a mixture of 200 nmol of 5′ end sequence and 400 nmol of poly(L-lysine) before infection with VSV, as above (lane 7), or labeled with [^{35}S]methionine without any preliminary treatment (lane 8). Lane 1 shows radioactive molecular weight standards (phosphorylase B, 97,400; bovine serum albumin, 69,000; ovalbumin, 46,000; carbonic anhydrase, 30,000; lactoglobulin A, 18,367), and lane 10 shows VSV proteins as revealed by labeling L929 cells with [^{35}S]methionine 5 hr after infection with VSV at a moi of 10; M, NS, N, G, and L at the right of the gel indicate the position of VSV proteins; their molecular weights are, respectively, 34,000, 45,000, 50,000, 65,000, and 151,000.

poly(L-lysine) did not alter total protein synthesis in VSV-infected cells.

This last set of results confirms the antiviral activity of the conjugate, the necessity of coupling to observe such activity, the specificity of our product, and the absence of toxicity in the doses used on L929 cells.

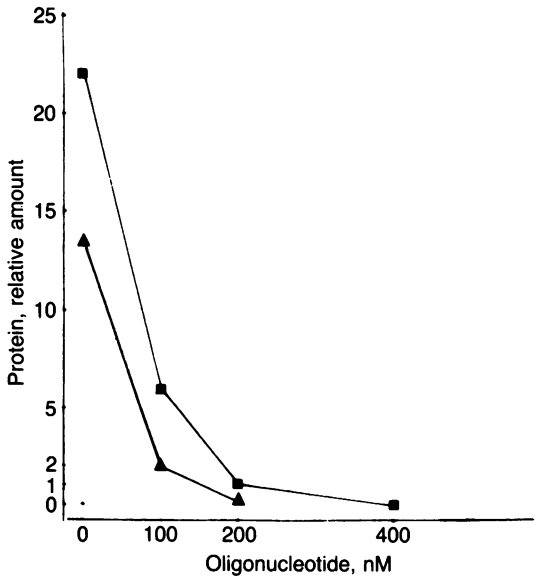

FIG. 4. Inhibition of VSV N and M protein synthesis by 5′ end oligodeoxyribonucleotide-poly(L-lysine) conjugate. Lanes 2–5 in Fig. 3 were scanned with a Vernon densitometer. The ratio of cellular protein p55 (indicated by the arrow on Fig. 3) to protein N (■) or protein M (▲) is recorded.

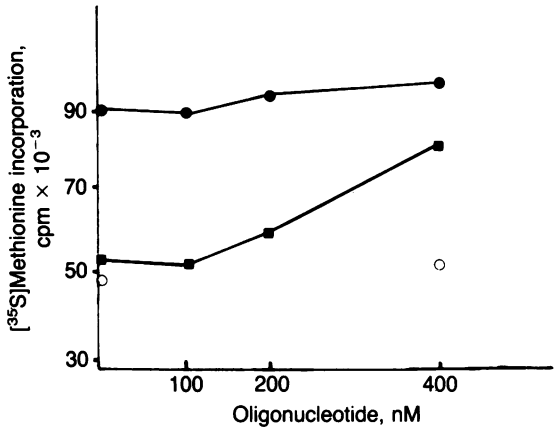

FIG. 5. Effect of oligodeoxyribonucleotide-poly(L-lysine) conjugate on protein synthesis in VSV-infected and uninfected L929 cells. L929 cells were incubated for 2 hr with different concentrations of 5′ end sequence poly(L-lysine) conjugate before infection (■) with VSV at a moi of 1 or in the absence of infection (●). As a control, L929 cells were incubated in the presence of poly(L-lysine) with or without 400 nmol of 5′ end sequence oligodeoxyribonucleotide (○). Cells were labeled with [^{35}S]methionine for 45 min, 6 hr after infection with VSV. Total acid-insoluble radioactivity was measured.

DISCUSSION

The results presented here demonstrate that a suitably chosen oligodeoxyribonucleotide sequence covalently conjugated at its 3′ end to an ε-amino residue of poly(L-lysine) promotes a specific and efficient antiviral activity against VSV in cultured cells. We were searching for a specific interaction of the conjugated oligonucleotide sequence with its intracellular RNA target—hence the choice of a 15-mer sequence, which should statistically not be found more than once in the mRNA population of infected or uninfected cells. Indeed, neither protein synthesis in uninfected cells nor viral protein synthesis in EMCV-infected cells was affected to a significant extent by the VSV-specific conjugate. The oligonucleotide sequence used in this study was designed to interact specifically with the initiation region of the mRNA coding for VSV N protein for maximal efficiency in translation inhibition. An oligonucleotide sequence complementary to an internal site of the same mRNA does not significantly alter VSV protein synthesis and VSV multiplication. Several explanations have been proposed for the increased efficacy of oligonucleotide sequences complementary to a translation initiation site compared to an internal site, such as (for instance) the accessibility to hybrid formation or the ability of 80S ribosomes to unfold mRNA secondary structure during elongation (34). Whatever the explanation(s), our experimental data agree with the results obtained in several other systems (3, 10, 18) and provide additional evidence for the specificity of the block in viral expression of our conjugates. This and the absence of effect of a mixture of the oligonucleotide sequence and poly(L-lysine) conclusively eliminate a possible trivial effect of oligonucleotide-poly(L-lysine) conjugates on the initial steps of VSV–cell interaction or on some cellular components indispensable for viral development.

The fact that an oligonucleotide complementary to VSV N protein mRNA and to none of the other VSV mRNAs inhibits to the same extent the synthesis of VSV M protein (and probably also of the other viral proteins) has been found by others (18) and does not arise from the lack of specificity. As for many viruses, efficient VSV multiplication requires an initial amplification of its genomic RNA; in addition, viral RNA must be coated with the nucleocapsid N protein to function as a template (35). A reduction in the translation of

N protein mRNA should therefore react on the accumulation of all five VSV proteins, as was observed. Alternatively, an oligonucleotide sequence complementary to one of the VSV mRNAs could also be hybridized, at least in principle, with the 40S plus-strand RNA serving as a template for the replication of virion RNA (36). This probably does not contribute to a large extent to the antiviral action and to the viral protein synthesis inhibitory activity of our conjugate since one would then have expected the oligonucleotide sequence complementary to an internal site of VSV N-protein mRNA to be as efficient as that of translation initiation.

Apart from its specificity, the inhibition of viral protein synthesis attained with oligodeoxyribonucleotide-poly(L-lysine) conjugates is remarkable for its efficiency as compared with data published so far in this field. The efficient inhibition of gene expression by antisense sequences in cell-free extracts or in intact cells usually requires concentrations of oligonucleotide sequences in the 50–500 μM range, although differences in the biological models investigated render comparisons difficult. Neutral methylphosphonate oligonucleotide derivatives (18) for instance have to be added at $\approx$100 times higher concentrations in the culture medium to reduce VSV multiplication in mouse L cells to a comparable extent as with poly(L-lysine) conjugates. This seemingly higher efficiency of poly(L-lysine) oligonucleotide conjugates could obviously result from several factors such as increased uptake and delivery to the appropriate cell compartment, better functional stability, or higher affinity for the target nucleotidic sequence. Too little is known at present about their metabolism, the pathway of their internalization, and their site of action in the cell to reach a conclusion, but several of these points are worth investigating further.

In summary, conjugation of properly located oligodeoxynucleotide sequence to poly(L-lysine) promotes a specific and efficient antiviral activity when the conjugates are incubated with cultured cells. This convenient and versatile tool should in principle make it possible to interfere specifically with the expression of any viral or cellular gene *in vitro* and possibly also *in vivo*. Moreover, the methodology used here to link an oligonucleotide sequence to ε-amino residues of lysine in poly(L-lysine) could conceivably be used for coupling them to amino groups in other synthetic or natural polypeptides, thus allowing targeting of the conjugated sequence to cells or tissues bearing specific cell-surface determinants.

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