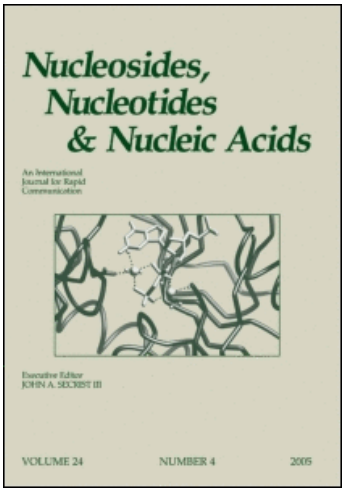

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Biological Activities of Oligonucleotides Linked to Poly(L-Lysine)

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To cite this Article Lemaltre, M. , Bisbal, C. , Bayard, B. and Lebleu, B.(1987) 'Biological Activities of Oligonucleotides Linked to Poly(L-Lysine)', Nucleosides, Nucleotides and Nucleic Acids, 6: 1, 311 — 315
To link to this Article: DOI: 10.1080/07328318708056211
URL: http://dx.doi.org/10.1080/07328318708056211

NUCLEOSIDES & NUCLEOTIDES, 6(1&2), 311-315 (1987)

BIOLOGICAL ACTIVITIES OF OLIGONUCLEOTIDES LINKED TO POLY(L-LYSINE)

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Abstract : We have developped a method to couple oligonucleotides to poly(L-lysine). This tool has been tested with anti m-RNA synthetic oligodeoxyribonucleotides and 2'-5'$(A)_n$ and allows oligonucleotides to enter intact cells.

Many oligonucleotides have been reported to play important roles in biological processes. The problem of handling these macromolecules is of a technical order : how can such oligonucletides be introduced into many cells nontraumatically since their high molecular weight and polarity hinders free diffusion across cellular membrane. A further problem is their poor stability in biological fluids.

Introduction of oligonucleotides into intact cultured cells by calcium phosphate precipitation, cell permeation or microinjection with micropipettes was successful in many cases. However, these drastic techniques cannot be extrapolated to the in vivo experimental work which will be required both for study of the role of individual oligonucleotides and for their possible use in chemotherapy.

Alternative intracellular delivery techniques have been tested for oligonucleotides such as : neutral oligonucleotide analogues (1,2), acridine conjugated oligonucleotides (3,4) or liposomes (5). We have devised an alternative method using poly(L-lysine) for the delivery of oligonucleotides to intact cells. Poly(L-lysine) has been described as a versatile macromolecular carrier (6) enabling the efficient intracellular

transport of somewhat different molecules such as proteins (7) or drugs (8) that do not normally penetrate cells.

Oligonucleotides can be covalently linked to poly(L-lysine) via a N-morpholine ring after periodate oxidation of their 3'-terminal ribose residue. We had to add an oxidizable 3'-terminal ribose in order to use this method with synthetic oligodeoxyribonucleotides, this operation can be performed with T4 RNA ligase (9). Two kinds of oligonucleotides have been tested (9,10). (fig. 1)

Naturally occuring RNA sequences which negatively control the expression of certain genes have been discovered recently in prokaryotes (11 for a recent review) ; such repressor activity probably arises from specific hybridization of short transcripts to complementary sequences in mRNAs giving terminology such as mic RNA (mRNA interfering complementary RNA (12) or "antisense-RNA" (13)).

We have thus synthesized a 15-mer oligodeoxyribonucleotide complementary to the ribosome binding site and initiation codon of the m-RNA coding for the N-protein of vesicular stomatitis virus (VSV). This oligodeoxyribonucleotide was coupled to poly(L-lysine) (M_r = 14,000) (9).

The incubation of L929 cells with this conjugate promotes dose-dependent inhibition of viral protein synthesis and dose-dependent reduction of VSV yield (Tab.1) (> 2 log at 400 nM of oligodeoxyribonucleotide in the incubation medium). The same conjugate neighter affects the synthesis of cellular proteins in uninfected cells nor exerts an antiviral activity on encephalomyocarditis virus. Furthermore, a 13-nucleotide long oligonucleotide complementary to an internal sequence of mRNA coding for the VSV N protein had no effect on VSV production. These results demonstrate the specificity of inhibition (9).

The inhibition of viral expression attained with these conjugates is remarkable for its efficiency, as compared with data published so far in this field. For instance, methylphosphonates oligonucleotide derivatives (14) have to be added to the culture medium at a concentration roughly one hundred times higher to reduce VSV production to the same extent as poly(L-lysine)-oligonucleotide conjugate.

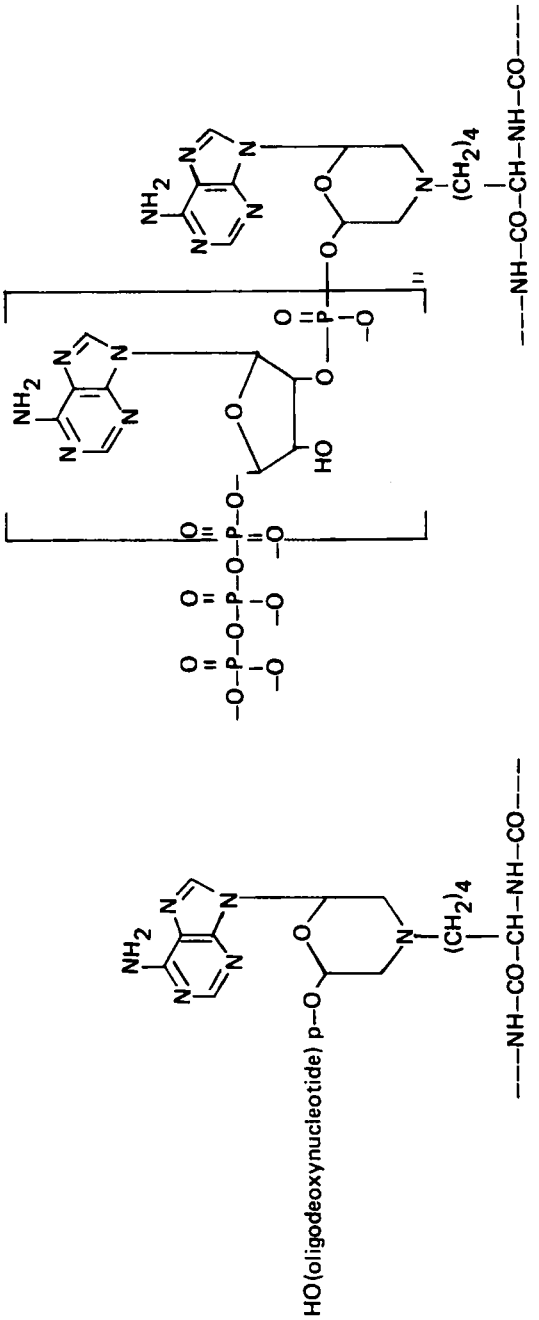

FIGURE 1

Tab. 1 ANTIVIRAL ACTIVITY OF OLIGONUCLEOTIDE-POLY(L-LYSINE) CONJUGATES

Oligonucleotide concentration (nM)	5'-end sequence coupled	5'-end sequence non coupled	5'-end sequence coupled	internal sequenc coupled
	VSV Titer	VSV Titer	EMCV Titer	VSV Titer
0	$4.2\ 10^8$ (100%)	$3.1\ 10^8$ (100%)	$5.8\ 10^7$ (100%)	$1.0\ 10^8$ (100%)
100	$1.7\ 10^8$ (40%)	$2.3\ 10^8$ (75%)	$3.1\ 10^7$ (53%)	$7.8\ 10^7$ (78%)
200	$7.8\ 10^6$ (2%)	$4.2\ 10^8$ (135%)	$3.1\ 10^7$ (53%)	$7.8\ 10^7$ (78%)
400	$3.0\ 10^6$ (0.7%)	$4.2\ 10^8$ (135%)	$5.8\ 10^7$ (100%)	$7.8\ 10^7$ (78%)

Tab. 2 BIOLOGICAL ACTIVITIES OF 2'-5'(oligoadenylate)$_4$ LINKED TO POLY(L-LYSINE)

	Poly(L-lysine) concentration	2'-5'$(A)_4$ concentration	VSV (a) Virus titer (%)	protein (b) synthesis (
poly(L-lysine) alone	500 nM	-	100%	100%
poly(L-lysine)+ 2-5A mixture	500 nM	1 uM	100%	100%
poly(L-lysine)-2-5A conjugate	500 nM	2.5 uM	1%	10%
	180 nM	900 nM	0.6%	10%
	100 nM	500 nM	5%	50%
	36 nM	180 nM	100%	90%

(a)- infection with VSV 3 hrs after addition of sample in culture medium
(b)- labelling (^{35}S-methionine) 3 hrs after addition of sample in culture medium

Another set of oligonucleotides - 2'-5'(oligoadenylates)- is naturally synthesized in cells treated with interferon. In the presence of double-stranded RNA or viral infection, 2'-5'$(A)_n$ binds to and activates endogenous RNase L which leads to transient RNA degradation, transient protein synthesis inhibition and an antiviral activity. 2'-5'$(A)_n$ do not naturally enter cells and is rapidly degradated. Once coupled

with poly(L-lysine) as outlined previously (10), 2'-5'$(A)_n$ conjugates conserves their ability to bind RNAse L. Moreover, the conjugate induces all the activities above when incubated with intact L1210 cells, while free 2'-5'$(A)_n$ has no such effects (10). (Tab. 2.)

Those results established for the first time that poly(L-lysine) could be used as an efficient carrier for small oligonucleotides of biological interest. This derivatization also protects oligonucleotides against degradation by phosphodiesterases.

It is now possible to carry out further studies on other viral or cellular genes (e.g. oncogenes, regulatory genes) and other peptidic carriers (e.g. neoglycoproteins) allowing targetting of the molecular hybrids on cells or tissues bearing particular surface determinants.

This work has been supported by grants from CNRS and INSERM. M.L. is the holder of a long term EMBO fellowship.

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