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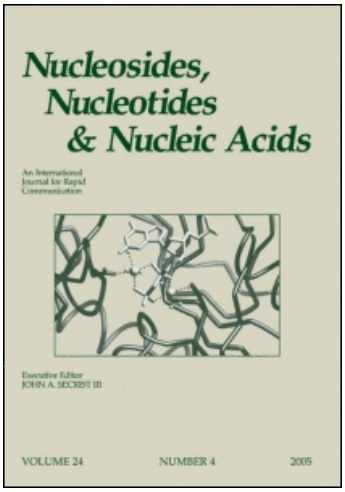

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Synthesis, Specific Intracellular Delivery and Biological Activity of Novel Stabilized (2'-5')$(A)_n$ Analogues

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NUCLEOSIDES & NUCLEOTIDES, 4(1&2), 157-160 (1985)

SYNTHESIS, SPECIFIC INTRACELLULAR DELIVERY AND BIOLOGICAL ACTIVITY OF NOVEL STABILIZED (2'-5')$(A)_n$ ANALOGUES

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SUMMARY

5' and 2' stabilized (2'-5')$(A)_n$ analogues were synthesized by chemical modifications of enzymatically polymerized (2'-5')$(A)_n$ oligomers. They exhibit an increased antiviral activity after microinjection in HeLa cell cytoplasm in agreement with their augmented metabolic stability. Their specific in vitro delivery to mouse leukemia cells after encapsulation in targetted liposomes leads to a transient inhibition of protein synthesis and an antiviral activity.

Convincing evidence for a role of (2'5')$(A)_n$ oligomers as mediators of interferon's antiviral activity is now available (1,2 for reviews).

The utilisation of (2'-5')$(A)_n$ derivatives in antiviral chemotherapy might thus be considered provided :

1) the metabolic stability of natural (2'-5')$(A)_n$ oligomer is increased without affecting structural requirements for recognition of a 2-5A dependent endoribonuclease (RNase L), its only known target. In addition, construction of analogues whose degradation products themselves are biologically active or cytotoxic should preferably be avoided.

2) the association of these derivatives with vectors and/or chemical modifications allowing their penetration in intact cells.

Adenosine 5' triphosphate was enzymatically polymerized with 2-5A synthetase from IFN-treated HeLa cells. (2'-5')$(A)_n$ oligomers were separated by ion-exchange chromatography and chemically converted to phosphodiesterase-resistant derivatives by oxidation of the terminal ribose residue with Na periodate and reduction with Na borohydride, followed by mild acid hydrolysis. Since such 2'-protected analogues are still subject to degradation by cellular phosphatases (3) similar modification of (αS) (2'-5')$(A)_n$ analogues polymerized enzymatically from adenosine 5'-O (3-thiotriphosphate) has been performed.

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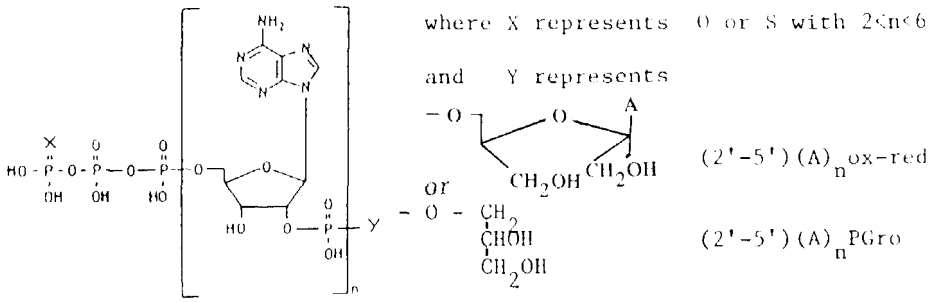

FIG.1 Analogues of (2'-5')(A)$_n$

(2'-5')(A)$_n$ analogues have been characterized by HPLC, ion exchange chromatography and RMN analysis.

The degradation of these 2' and/or 3' modified (2'-5')(A)$_n$ derivatives in cell-free extracts has been followed by HPLC (Fig.2).

(2'-5')(A)$_n$ derivatives protected at both their 5' and 2' ends are more stable than 2'end-modified analogues or unmodified (2'-5')(A)$_n$ oligomers in agreement with a contribution of both phosphodiesterases and phosphatases in (2'-5')(A)$_n$ catabolism.

(2'-5')(A)$_n$ derivatives compete to the same extent as natural (2'5')(A)$_n$ with a [^{32}P] (2'-5')(A)$_n$pCp probe for binding to RNase L They exhibit a powerful antiviral activity against vesicular stomatitis virus (VSV), an RNA virus, when microinjected with micropipettes in HeLa cell cytoplasm.

This and other work (1,4 for reviews) thus provides satisfactory means for the stabilization of (2'-5')(A)$_n$ oligomers against degradation by cellular enzymes. However no solution has yet been provided to the problems caused by cell impermeability towards such highly charged compounds. Indeed, reported biological activity of less charged dephosphorylated or "core" (2'-5')(A)$_n$ derivatives is not exerted through re-phosphorylation and activation of RNase L (5 for instance). Attempts to decrease the hydrophilic character of

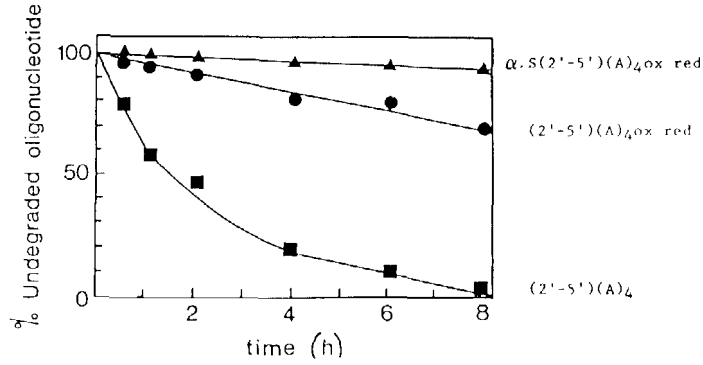

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TABLE 1 Antiviral activity of liposome-encapsulated (2'-5')(A)$_5$ and (2'-5')(A)$_5$ analogues

Cell treatment	Virus yield (infectious units x ml^{-1})	% control
Free (2'-5')(A)$_5$	3.6×10^7	-
Free (2'-5')(A)$_5$-PGro	1.8×10^7	-
Liposomes without (2'-5')(A)$_5$	2.8×10^7	100
Liposomes with (2'-5')(A)$_5$	1.8×10^7	63
Liposomes with (2'-5')(A)$_5$PGro	1.5×10^6	5

(2'-5')(A)$_n$ oligomers have been unsuccessful until now (6 for example) and presently described ways (cell permeabilisation, calcium phosphate coprecipitation or microinjection) of introducing (2'-5')(A)$_n$ in intact cells are not susceptible of in vivo extrapolation.

We therefore encapsulated (2'-5')(A)$_n$ or (2'-5')(A)$_n$ derivatives in the aqueous compartment of small unilamellar liposomes covalently coupled to Staphylococcus aureus protein A.

This provided a vehicle whose contents can be delivered specifically with the help of appropriate monoclonal antibodies (directed against murine histocompatibility antigens in our experimental model) to cells bearing the cognate antigens at their surface (7). Protein synthesis is inhibited in a time-dependent way after the interaction of mouse leukemic L1210 cells with (2'-5')(A)$_n$ - containing liposomes, as expected if the 2-5A dependent endoribonuclease is activated by the intracytoplasmic delivery of (2-5')(A)$_n$ oligomers after endocytosis of liposome-associated material. Such cells are partially protected against virus infection when challenged with VSV as illustrated in Table 1 for the (2'-5')(A)$_n$ PGro analogue.

Although several problems remain to be solved before any in vivo extrapolation, these experiments strongly suggest that stabilized (2'5')(A)$_n$ derivatives in association with suitable vectors are capable of entering cells through an endocytic pathway while keeping their biological activity.

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