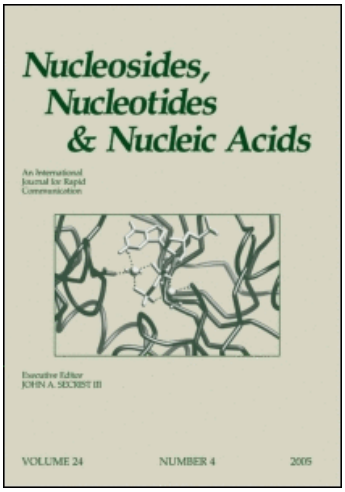

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

POLYIMIDAZOLE CONJUGATED OLIGONUCLEOTIDES REACH THE NUCLEUS OF HELA CELLS

François Morvan[a]; C. Castex[a]; E. Vivès[b]; Jean-Louis Imbach[a]
[a] Université Montpellier II, Montpellier, Cedex 5, France [b] Institut de Génétique Moléculaire UMR 5535 CNRS-UM II 1919, Montpellier, Cedex 5, France

Online publication date: 31 March 2001

To cite this Article Morvan, François , Castex, C. , Vivès, E. and Imbach, Jean-Louis(2001) 'POLYIMIDAZOLE CONJUGATED OLIGONUCLEOTIDES REACH THE NUCLEUS OF HELA CELLS', Nucleosides, Nucleotides and Nucleic Acids, 20: 4, 805 — 808
To link to this Article: DOI: 10.1081/NCN-100002434
URL: http://dx.doi.org/10.1081/NCN-100002434

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS, 20(4–7), 805–808 (2001)

POLYIMIDAZOLE CONJUGATED OLIGONUCLEOTIDES REACH THE NUCLEUS OF HELA CELLS

François Morvan,[1,*] C. Castex, E. Vivès,[2] and Jean-Louis Imbach[1]

[1]Laboratoire de Chimie Organique Biomoléculaire de Synthèse, UMR 5625 CNRS-UM II, Université Montpellier II, Place E. Bataillon, 34095 Montpellier Cedex 5, France
[2]Institut de Génétique Moléculaire UMR 5535 CNRS-UM II 1919, Route de Mende 34293 Montpellier Cedex 5, France

ABSTRACT

Oligonucleotide models bearing 6, 12 or 18 histamine residues were synthesized on solid support and labeled with fluorescein. Only the oligo with 6 histamine residues showed a high uptake in HeLa cells with a nuclear localization. Experiment a 4°C or with bafilomicyn A_1 suggest that uptake proceeded by an endocytosis mechanism followed by a destabilization of the membrane. Once in the cytoplasm the oligo reached rapidly the nucleus.

Oligonucleotide (ODN)-based therapy promises to be a highly specific tool for the treatment of numerous human diseases. However the effectiveness of ODNs has been mainly limited by poor nuclease resistance and poor cellular uptake. Nuclease resistance was greatly increased by the use of phosphorothioate ODNs but they still displayed limited cellular uptake. Furthermore by endocytosis the ODNs are entrapped in endosomes and are not available to interact with their targets. Recent publications pointed up the high uptake of histidyl-rich peptide (1) and the use of histidylated oligolysines to increase the uptake of ODN (2). Histidyl residues are described to destabilize membrane after protonation of the imidazole group and allow the escape of the ODN from the endosomes.

*Corresponding author.

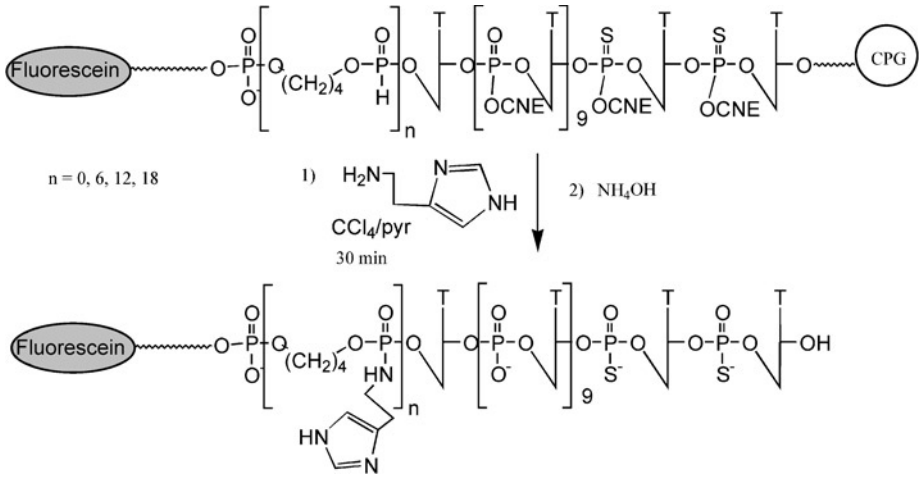

Figure 1. Synthesis the polyimidazoyl T_{12} models by amidative oxidation of H-phosphonate linkages.

We present the synthesis of dodecathymidine models covalently link to several histamine residues and their uptake in HeLa cells. Three T_{12} models bearing an increase number of imidazole groups (6, 12 and 18) were synthesized on solid support. Imidazole residues were introduced into a fluorescein labeled T_{12} models by amidative oxidation of H-phosphonate linkages with histamine carbon tetrachloride (Fig.1). As control the fluorescein labeled T_{12} was also synthesized (Fig.1, $n = 0$).

The resulting oligos were characterized by MALDI-TOF Mass spectrometry. Mass spectra showed that amidative oxidation was incomplete with the presence of H-phosphonate links oxidized into phosphodiester links. For the $FluoHis_6T_{12}$, mass spectra displayed peaks corresponding to $n = 6$ (in majority) $n = 5$ (less abundant) and $n = 4$ (small amount). For the two other oligos, mass spectra displayed a gaussian distribution centered at $n = 10$ and $n = 15$ for $FluoHis_{12}T_{12}$ and $FluoHis_{18}T_{12}$ respectively. To study the behavior of the imidazole group linked to an oligo it was not necessary to use pure oligos, thus the oligos were used without purification.

The crude oligos, dissolved in OptiMEM serum free medium, were incubated at a 10 μM concentration with HeLa cells for 2 h at 37°C. A post incubation treatment with monensin (50 μM) at 4°C for 30 min was necessary to detect the fluorescence into the cells. Indeed monensin induces the neutralization of all acidic compartments and reveals the fluorescein from quenching (3). A high uptake was found for the $FluoHis_6T_{12}$ while the $FluoHis_{12}T_{12}$ was less taken up. Finally when exposition time was increased 10-fold a slight fluorescence was visualized for the $FluoHis_{18}T_{12}$ and the control $FluoT_{12}$. Hence the $FluoHis_{18}T_{12}$ displayed only a low uptake but still higher than the control $FluoT_{12}$ (Fig. 2). Fluorescence was strongly localized in nucleus and slightly in the cytoplasm. No punctuation was visualized in the cytoplasm suggesting that oligos were not entrapped into endosomes.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

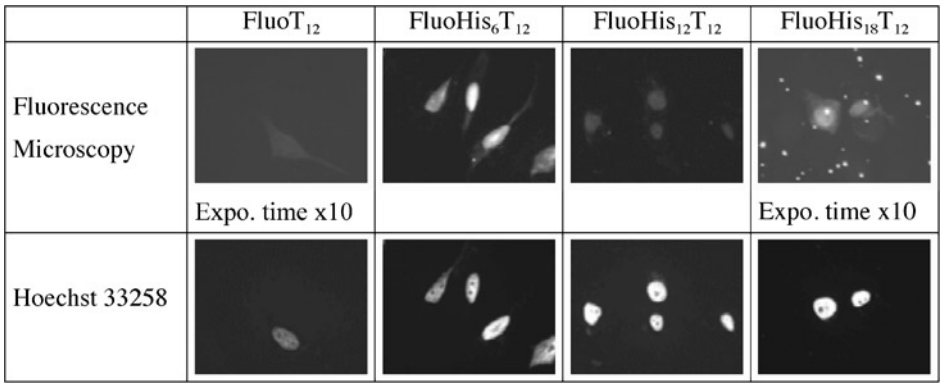

Figure 2. Fluorescence microscopy of fluorescein labeled polyimidazole T_{12} models and visualization of the HeLa cell nucleus by Hoechst 33258 dye.

Furthermore, nuclear localization implies that oligos were found free in the cytoplasm. The decreasing uptake for oligos bearing an higher contents of histamine residues could be tentatively explained by a too high molecular weight of the resulting oligos.

Incubations were repeat at 4°C and a dramatic decrease of uptake was found (Fig. 3) suggesting that uptake proceeded through an active transport likely by endocytosis. Finally the oligos were incubated at 37°C for 2 h with HeLa cells in presence of bafilomicyn A_1. Bafilomicyn A_1 is known to inhibit the proton pump ATPase (4) involved in the endosome acidification. Under these conditions, the fluorescence was not visible into HeLa cells for any of the oligos. This result indicates that protonation of the imidazole residues is involved in the transmembrane passage of the ODN.

The hybridization of the polyimidazole T_{12} models with poly rA was studied by melting curve in 10 mM sodium cacodylate, 1 M sodium chloride pH 7 as buffer. All the duplexes displayed the same Tm around 41°C indicating that the 5′-polyimidazole tail has not effect on the duplex formed.

An oligo model dodecathymidine with only 6-histamine residues was efficiently taken up by HeLa cells. The uptake of this oligo proceeded likely by

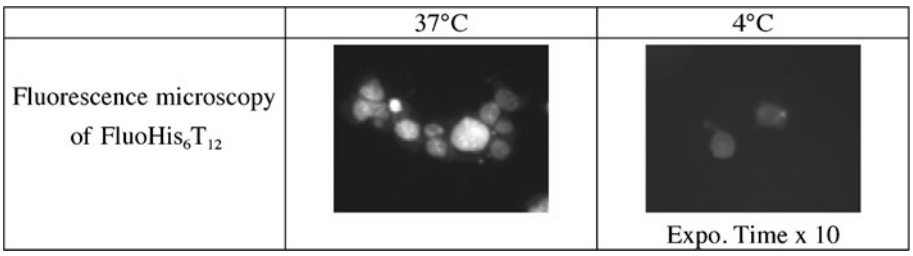

Figure 3. Fluorescence microscopy of $FluoHis_6T_{12}$ incubated with HeLa cells at 37°C and 4°C.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

endocytosis then the imidazole group after protonation allowed the oligo to escape, once in the cytoplasm it reached rapidly the nucleus. This efficient method to increase the uptake could be easily extend to any oligonucleotide.

ACKNOWLEDGMENTS

This work was supported by grants from "Association pour la Recherche contre le Cancer" (ARC) and the "Comités de l'Aude et des Pyrénées Orientales de la Ligue contre le Cancer".

REFERENCES

1. Midoux, P.; Kichler, A.; Boutin, V.; Maurizot, J. C.; Monsigny, M. *Bioconjugate Chem.* **1998**, *9*, 260–267.
2. Pichon, C.; Roufai, M. B.; Monsigny, M.; Midoux, P. *Nucleic Acids Res.* **2000**, *28*, 504–512.
3. Maxfield, F. R. *J. Cell. Biol.* **1989**, *95*, 676–681.
4. Bowman, E. J.; Siebers, A.; Altendorf, K. *Proc. Natl. Acad. Sci. USA* **1988**, *85*, 7972–7976.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Request Permission or Order Reprints Instantly!