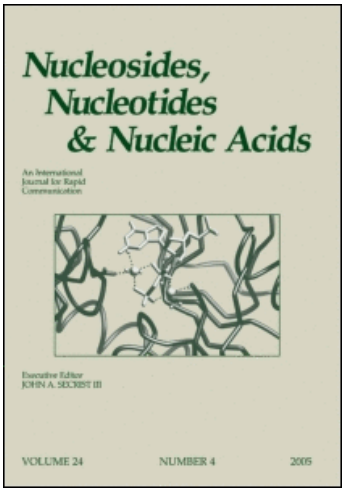

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J. -R. Bertrand[a]; N. Sumbatyan[b]; C. Malvy[a]
[a] CNRS, UMR 8121, Institut Gustave Roussy, Villejuif cedex, France [b] Chemistry Department of Moscow State University, Moscow, Leninskie gory, Russian Federation

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Covalent Coupling of a PIM-1 Oncogene Targeted PNA with an Antennapedia Derived Peptide

J.-R. Bertrand,[1,*] N. Sumbatyan,[2] and C. Malvy[1]

[1]CNRS, UMR 8121, Institut Gustave Roussy, Villejuif cedex, France
[2]Chemistry Department of Moscow State University, Moscow, Leninskie gory, Russian Federation

ABSTRACT

Peptide nucleic acids (PNA) are promising antisense molecule for blocking gene expression in cell culture or in vivo. Nevertheless because they are poor efficient to pass the cellular membrane, it is necessary to use a vectorisation agent to observe an inhibitory effect. We describe the coupling of the rhodamine labeled 17-mer antisense PNA to a fusogenic peptide from antenapedia via S-S linkage, the studies of the penetration of this complex into fibroblast cells and its inhibitory effect on piml targeted protononcogene.

Key Words: PNA; Antenapedia peptide; Penetratin; Cell penetrating peptide; Covalent conjugate; Oncogene inhibition; Pim-1.

Peptide nucleic acids (PNA), oligonucleotide mimics possessing non-charged achiral polyamide backbone modified with nucleobases, are extremely stable in biological fluids. They specifically hybridize to nucleic acids in a complementary manner, forming very strong heteroduplexes, and consequently can inhibit gene transcription and translation by tight binding to either DNA or RNA.[1] Progress

*Correspondence: J.-R. Bertrand, CNRS UMR 8121, Institut Gustave Roussy, 39 rue Camille Desmoulins, 94805 Villejuif cedex, France; E-mail: irb@igr.fr.

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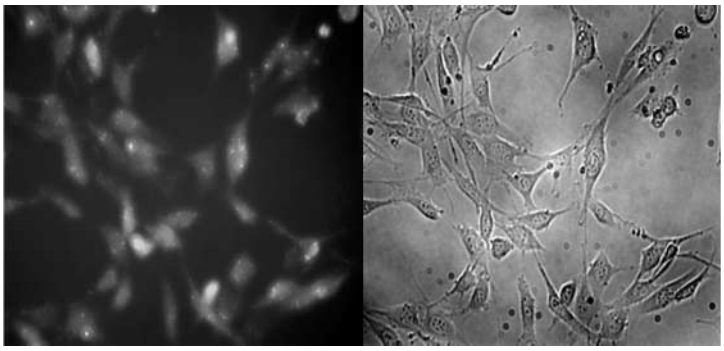

Figure 1. Internalization of the Rho-PNA-S-S-penetratin-Bio by NIH 3T3 cells in DMEM containing 10% fetal bovine serum. The oligonucleotide was incubated for 12 h at 0.8 μM concentration.

in the use of PNA as an antisense reagent was hampered by their poor cellular uptake.[2] It was shown that the most efficient uptake was obtained when PNA oligomers were coupled to cell penetrating peptides, such as the 16 amino acid homeodomain of the Antennapedia protein from Drosophila (penetratin).[3] We have used an antisense PNA targeted against the pim-1 oncogene mRNA in murine cells. Here we report the preparation of this rhodamine labeled 17-mer antisense PNA covalently linked with biotinylated penetratin via S-S linkage, the studies of its cellular uptake and the first results about its inhibitory effect on the protooncogene pim-1 mRNA translation.

The 17-mer PNA labeled with rhodamine at N-terminal and containing a cysteine residue at its C-terminal was coupled with biotinylated penetratin bearing an active pyridyl disulfite function at its N-terminal end. A preliminary reduction of the thiol function of the PNA oligomer was carried out. Equimolar amounts of

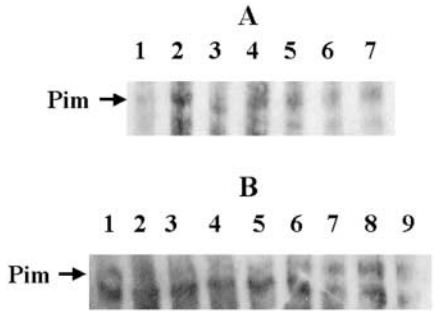

Figure 2. Western Blot measurement of the Pim-1 protein in NIH 3T3 cells after 24 hours incubation. The autoradiogram after 12% SDS-polyacrylamide gel transfer on a nitrocellulose followed by the immunodetection and visualization is shown. A: lane 1– control without proteins; lane 2 – control with Pim antibodies; lanes 3,4 – Rho-PNA; lanes 5–7 – Flu-PNA. B: lane 1– a control with pim antibodies; lanes 2–4 – Rho-PNA-S-S-penetratin-Bio; lanes 5,6 – oligonucleotide 5 (antisense); lanes 7–9 – oligonucleotide 6 (control).

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the both oligomers were incubated for 12 h at 37°C and then product was purified on Sephadex G-50 and by HPLC.

NIH 3T3 cells were used to study the cellular uptake and the inhibitory effect of the synthesized conjugate on the pim-1 protooncogene mRNA translation. Cells were incubated with 800 nM solution of the conjugate or in DMEM containing 10% fetal bovine serum or in OPTIMEM for 12 h. Visualization by fluorescent microscopy indicated that the 17-mer PNA labeled with rhodamine coupled with biotinylated penetratin efficiently penetrated into NIH 3T3 cells, moreover some more efficient internalization was observed in DMEM containing 10% fetal bovine serum (Fig. 1). The cellular localization of the conjugate in NIH 3T3 cells was monitored using confocal microscopy. Visualization in living cells demonstrated that most of the conjugate is localized at the cytoplasmic membrane and only a small amount penetrates into intracellular compartments and could be then responsible for a biological effect.

Pim-1 protein synthesis inhibition in NIH 3T3 cells was followed by Western Blot using an antibody targeted against pim-1 protein. Taking into account the low pim-1 expression in NIH 3T3, we had to use 1 million cells per well. Our first results show that the PNA-penetratin conjugate strongly reduces the pim-1 protein expression at an extracellular concentration of 400 nM (Fig. 2) without any obvious cell toxicity.

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