

CPP or Cholesterol Conjugated Antisense PNA for Cellular Delivery

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Peptide nucleic acid (PNA) is a DNA mimic consisting of the four common bases of DNA on a pseudopeptide backbone that makes it extremely stable in biological fluids. Antisense PNA is targeted against mRNA in cytoplasm in a sequence specific manner. However, the main hindrance to the effective use of PNAs has been their relatively poor uptake by cells. Endosomal release or direct uptake into cytosol of agents is mandatory for attaining mRNA based targeting. There are reports on the cell penetrating peptide (CPP) based delivery system. It has also been reported that conjugates of cholesterol and siRNAs facilitate cellular import [1]. The aim of this study was to synthesize different sequences of cholesterol coupled antisense PNA and to compare its uptake characteristics with a CPP-PNA conjugate.

The synthesis of PNA (anti-dsRed PNA (agcgcctgtacc), specifically targeted to mRNA of dsRed, a red fluorescent protein) conjugated to CPP (d-Tat) or cholesterol was performed in fully automated synthesizer (Prelude, Protein Technologies, Inc.) using continuous solid phase chemistry. To increase the solubility in water, linkers (AEEA) and additional charged amino acids were coupled or the sequence of peptide, PNA and cholesterol was changed. All compounds were labelled with FITC to confirm the cellular uptake by fluorescence microscopy and spectroscopy.

Cell uptake studies showed that the CPP bound PNA was located predominantly in vesicles indicating an endosomal uptake mechanism and subsequent entrapment in vesicles. Cholesterol bound PNA was also efficiently internalized. However, it was also located inside vesicles without detectable cytosolic distribution.

PNA-Cholesterol has fewer synthetic steps than PNA-CPP. However, it was also located inside vesicles restricting its applicability for mRNA targeting. The efficient uptake might make it a promising cellular delivery agent after further improvements.

[1] C. Wolfrum *et al.*, *Nature Biotechnology*, 2007, 25, 1149