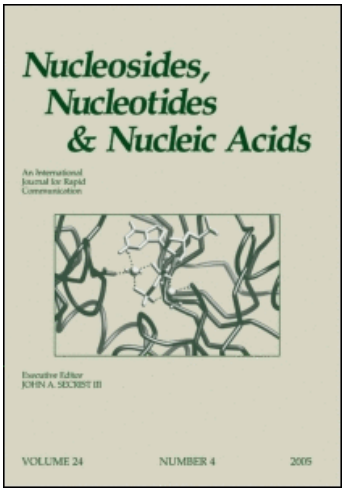

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Linear Polyethylenimine as a Tool for Comparative Studies of Antisense and Short Double-Stranded RNA Oligonucleotides

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Linear Polyethylenimine as a Tool for Comparative Studies of Antisense and Short Double-Stranded RNA Oligonucleotides

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ABSTRACT

Despite the recently enlarged field of available RNA knock-down technologies, e.g., antisense oligonucleotides (ASOs) and duplexes of synthetic 21 nucleotides RNAs (siRNAs), no versatile transfection reagent has been reported to deliver different nucleic acids formats at high rates of efficiency. We have evaluated the versatility and efficacy of linear PEI in transfecting and properly delivering a broad panel of nucleic acids such as short oligonucleotides and double-stranded RNA into cells in culture.

Antisense oligonucleotides (ASOs) have become powerful tools in modulating genes and thereby contributing to the elucidation of their function and putative role in disease processes. Recently, duplexes of synthetic 21 nucleotides RNAs (siRNAs) have been demonstrated to be efficient tools to suppress genes in a variety of different mammalian cell lines by a mechanism called RNA interference (RNAi) offering

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additional options for the manipulation of gene expression (1). However, a key challenge with regard to automation of screening synthetic oligonucleotides in mammalian systems is a reliable method for delivering an effective concentration of these compounds into the cytosol/nuclear compartment of cells. Until now, to our knowledge no universal transfection reagent has been reported to deliver different nucleic acid formats at equal rates of efficiency.

Among the various nucleic acid carriers that have been described, polyethylenimines (PEIs) have indicated promising efficacy in cell culture transfections as well as in a variety of in vivo applications (2). While several essential features necessary for efficient transfection, such as DNA condensation, protection from nucleases and endosomal release are already intrinsic to PEI molecules, additional chemical modifications can be easily introduced to improve target specificity as well as biocompatibility for in vivo studies. The studies described here were aimed at the assessment of linear PEI as a universal carrier for different nucleic acid derivatives such as modified ASOs and siRNA in vitro.

In our hands, PEI complexation with partially or fully modified ASOs and even full DNA phosphorothioate oligomers revealed a high and specific target down-regulation. As expected, under comparable conditions, no significant antisense activity was observed for an unmodified phosphodiester ASO (data not shown).

As an alternative to the lipid formulations commonly described in the literature, we also investigated the capability of linear PEI to deliver short RNA duplexes (siRNAs) in vitro. A ratio of N/P = 5 (nitrogen/phosphate ratio) was found to be optimal for siRNA activity, whereas for N/P = 3 slightly lower levels of mRNA down-regulation were observed (Fig. 1A). PEI-mediated transfection of siRNA induced mRNA down-regulation also at a relatively low siRNA concentration of 50 nM (Fig. 1B) with comparable efficacy to conventional transfection procedures applying oligofectamine.

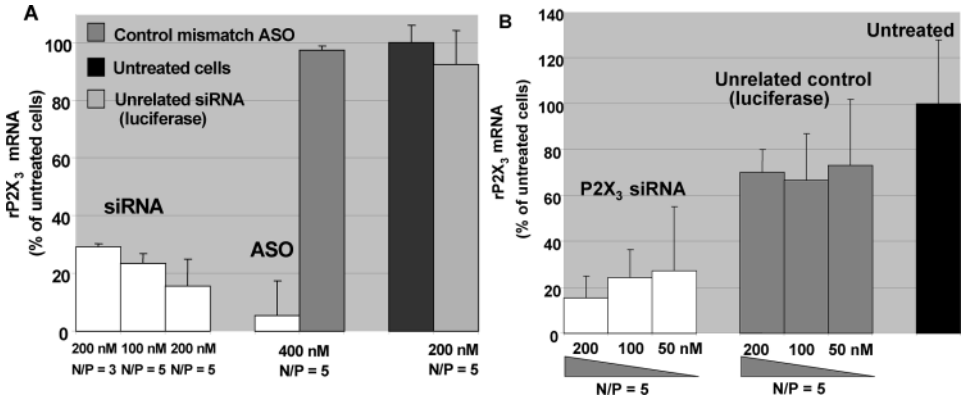

Figure 1. PEI-mediated inhibition of rat $P2X_3$ mRNA by transfection of a short (21 nt) $P2X_3$ siRNA in a stably transfected Chinese Hamster Ovary cell line expressing recombinant rat P2X3 cDNA sequence (3). A: at N/P ratios of 3 and 5 (100 and 200 nM) compared with an antisense match and mismatch oligonucleotide at 400 nM; B: siRNA-mediated dose-dependent repression of $P2X_3$ mRNA.

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Regarding the application of ASOs and siRNAs as gene knock-down reagents in vitro, we have demonstrated the capability of linear PEI to efficiently deliver different modified antisense compounds as well as siRNA duplexes into a mammalian cell line and induce potent target down-regulation comparable to common nucleic acid transfection protocols. To our knowledge, this is the first study employing a cationic polymer to efficiently transfect siRNA, providing attractive advantages over cationic lipid formulations such as an easier access to chemical modifications of the carrier. The versatility of linear PEI makes it an attractive formulation for studying chemical modifications and head-to-head comparison between RNA knock-down tools such as antisense oligonucleotides and siRNAs under the same experimental procedures and cellular delivery conditions.

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