Journal of Biotechnology 227 (2016) 103–111

Contents lists available at ScienceDirect

Journal of Biotechnology

journal homepage: www.elsevier.com/locate/jbiotec

Comparative study of polyethylenimines for transient gene expression in mammalian HEK293 and CHO cells

Laurence Delafosse[a,b], Ping Xu[b], Yves Durocher[a,b,*]

[a] Département de biochimie et médecine moléculaire, Faculté de Médecine, Université de Montréal, QC, Canada
[b] National Research Council of Canada, Building Montreal-Royalmount, 6100 Royalmount Avenue, Montreal, QC H4P 2R2, Canada

ARTICLE INFO

Article history:
Received 24 December 2015
Received in revised form 30 March 2016
Accepted 12 April 2016
Available online 13 April 2016

Keywords:
Acylation
Polyplex
Attachment
Internalization
Transfection
Recombinant protein

ABSTRACT

Three commercially available linear polyethylenimines (25 kDa LPEI, 40 kDa PEI"Max" and PEIpro™) were compared regarding their potency to transfect serum-free growing and suspension-adapted HEK293 and CHO cells. We determined the optimal DNA:PEI ratios for maximal expression of the reporter gene SEAP while monitoring cytotoxicity following transfection. PEIs acylation was determined by ^{1}H NMR and their apparent size and polydispersity assessed by size-exclusion chromatography. The propensity of PEIs to condense plasmid DNA was evaluated by agarose-gel electrophoresis. The zeta potentials and particle sizes at optimal DNA:PEI ratio were analyzed. Polyplex attachment to the cells and internalization kinetics were monitored. The quantity of PEIpro™ needed to efficiently transfect the cells was significantly lower than with LPEI and PEI"Max" and, interestingly, the maximal amount of internalized PEIpro™-based polyplexes was approximately half of that observed with its counterparts. PEIpro™ was the largest and least polydisperse polymer, but also the most cytotoxic. The optimal transfection conditions were subsequently used to express three monoclonal antibodies at larger-scale. The use of the deacylated PEI"Max" and PEIpro™ resulted in a significant increase of recombinant protein expression compared to LPEI. These findings demonstrate the importance of properly choosing the most suitable polymers to obtain optimal recombinant protein transient expression.

1. Introduction

Large-scale transient gene expression (TGE) in mammalian cells is widely used for the fast production of recombinant proteins (r-proteins) that require proper folding, assembly and post-translational modifications for their biological activities. TGE can generate milligram to gram quantities of r-proteins within a few days (Baldi et al., 2007; Geisse and Fux, 2009; Pham et al., 2006) and thus supersedes the long, tedious and costly process associated with the establishment of high-producing stable cell lines. CHO (Chinese hamster ovary) and HEK293 (human embryonic kidney 293) cell lines have been successfully used as transient expression systems. While transfection of serum-free adapted and suspension-growing mammalian cells can be performed by a variety of transfection reagents (Chahal et al., 2011), polyethylenimine (PEI), a cationic polymer, is probably the most widely used, characterized and cost-effective reagent for large-scale applications (Geisse and Fux, 2009; Pham et al., 2006).

Linear PEI (LPEI) with an average molecular weight of 25 kDa is routinely used for TGE because it exhibits high transfection efficiencies and low cytotoxicity (Aravindan et al., 2009; Dai et al., 2011; Nimesh et al., 2007). LPEI contains residual N-propionyl groups that reduce the number of protonatable nitrogen residues and as such may limit nucleic acid condensation and endosomal escape (Thomas and Klibanov, 2002). Indeed, nearly fully deacylated LPEIs have been developed and shown to be superior for mammalian cell transfection (Jeong et al., 2001; Thomas et al., 2005). Commercially available deacylated PEIs are known as PEI"Max" (Polysciences) and PEIpro™ (Polyplus Transfection). The impact of LPEI acylation levels on transfection efficacy has however shown contradictory results (Forrest et al., 2004; Gabrielson and Pack, 2006; Kadlecova et al., 2012).

Improvement of large-scale mammalian cell transfection systems can be achieved through fine-tuning multiple parameters such as the expression vector, transfection reagent, cell line, culture medium and feed addition post-transfection. The proper selection of the transfection reagent thus represents an easy parameter to optimize and may have significant impact on TGE process. With

* Corresponding author at: National Research Council of Canada, Building Montreal-Royalmount, 6100 Royalmount Avenue, Montreal, QC H4P 2R2, Canad National Research Council of Canada, Montreal, QC, Canada.
E-mail address: yves.durocher@cnrc-nrc.gc.ca (Y. Durocher).

http://dx.doi.org/10.1016/j.jbiotec.2016.04.028
0168-1656/

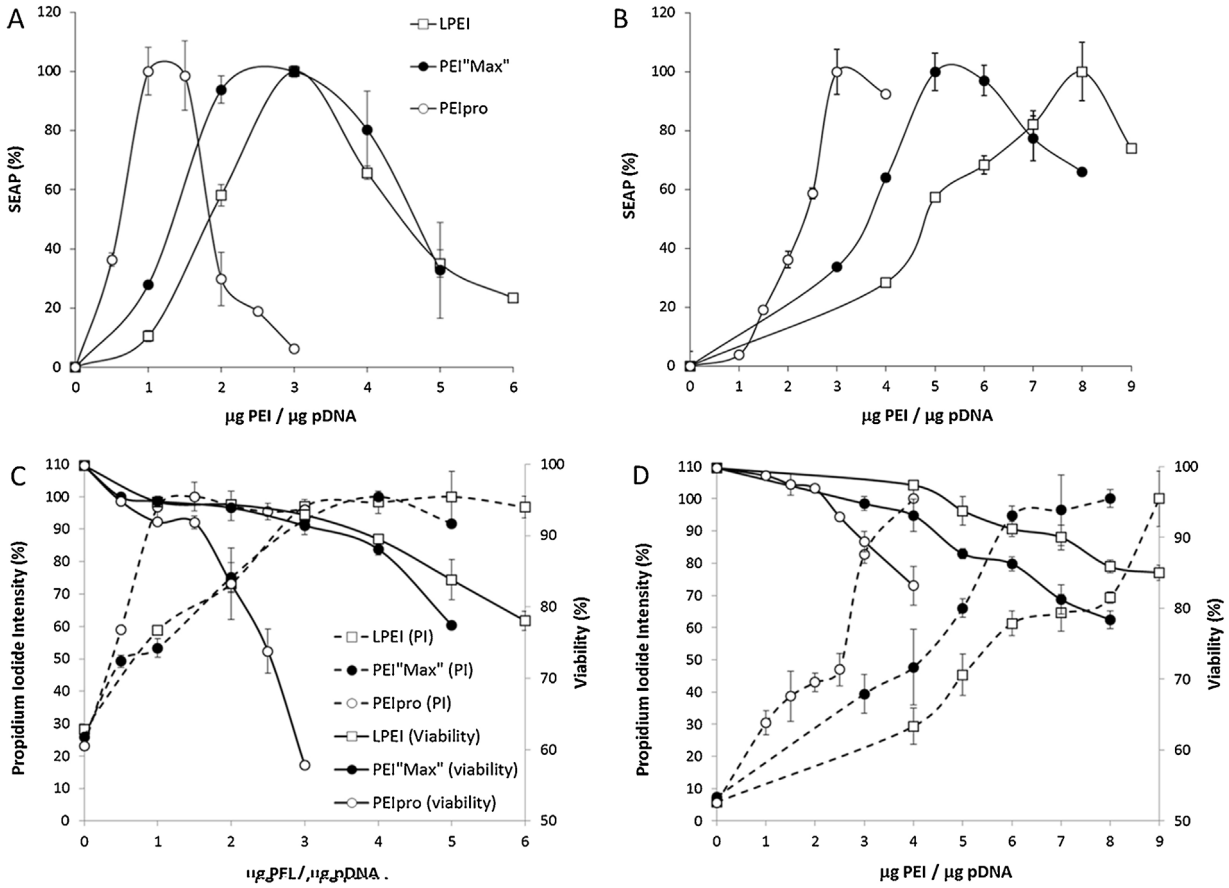

Fig. 1. PEIs-mediated transfection of HEK293 and CHO cells, propidium iodide labeling and viability determination. Increasing concentrations of polymers were used to form polyplexes using 1 µg of plasmid DNA. The SEAP activity present in the culture medium from HEK293 (A) and CHO (B) cells was determined at day 6 post-transfection and reported as percentage of maximal SEAP expression for each polymer. Propidium iodide labeling was used to measure the membrane integrity of HEK293 (C) and CHO (D) cells 48 hpt. Viability (as determined by flow cytometry analyses) of transfected HEK293 (C) and CHO (D) cells was determined at 48 hpt. Results were normalized to controls without transfection and are representative of three independent experiments conducted in triplicate.

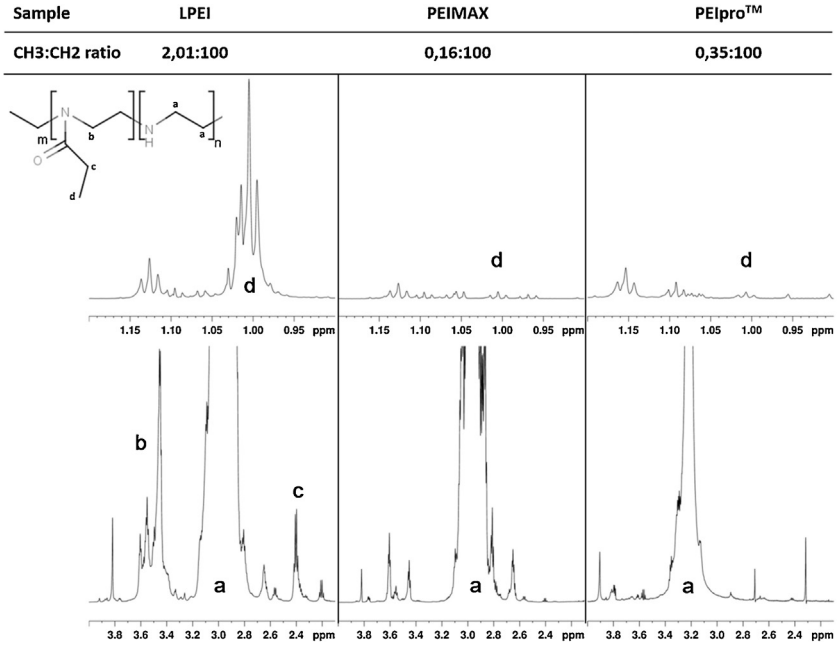

Fig. 2. 1D ^{1}H NMR spectra of partially acylated LPEI and fully deacylated PEI"Max" and PEIpro™. a and b correspond to CH_2 residues in the PEI backbone while c and d correspond respectively to the CH_2 and CH_3 residues in the propionyl side-chain.