Journal of Controlled Release 154 (2011) 171–176

Contents lists available at ScienceDirect

Journal of Controlled Release

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

Poly(β-amino ester)–DNA complexes: Time-resolved fluorescence and cellular transfection studies

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ARTICLE INFO

Article history:
Received 9 February 2011
Accepted 4 June 2011
Available online 14 June 2011

Keywords:
Gene delivery
Poly(β-amino esters)
DNA complexation
DNA binding
Time-resolved fluorescence spectroscopy

ABSTRACT

A large number of different polymers have been developed and studied for application as DNA carriers for non-viral gene delivery, but the DNA binding properties are not understood. This study describes the efficiency of nanoparticle formation by time-resolved fluorescence measurements for poly(β-amino esters), cationic biodegradable polymers with DNA complexation and transfection capability. From the large library of poly(β-amino esters) ten polymers with different transfection efficacies were chosen for this study. The binding constants for nanoparticle formation were determined and compared to with the same method. Although the DNA binding efficiency of the amine groups are similar for both types of polymers, the overall binding constants are an order of magnitude smaller for poly(β-amino esters) than for 25 kDa polyethylenimines, yet poly(β-amino esters) show comparable DNA transfection efficacy with polyethylenimines. Within this series of polymers the transfection efficacy showed increasing trend in association with relative efficiency of nanoparticle formation.

1. Introduction

The viruses are able to transfer their genetic cargo to the host cells. Although most of current gene therapy research relies on viral vectors, safety problems (incl. deaths in clinical trials) have slowed down the progress of this approach [1–3]. Non-viral (chemical) vectors, based on nanoparticles, are potential alternatives to the viral vectors. They offer advantages that are difficult to achieve with viral vectors: versatility, lack of immunogenicity, easy large-scale production, unrestricted DNA size, and possibility to incorporate several different DNA species to the same particle [4]. However, the chemical vectors show poor transfection efficacy [5]. Typical non-viral DNA delivery systems involve polycationic species, like cationic polymers, cationic liposomes or micelles, that complex and condense plasmid DNA in solution forming nanoparticles of 40–500 nm in diameter [6–9]. Nanoparticle mediated gene transfection involves several phases: DNA complexation, binding to the cell surface, endocytic uptake, endosomal escape to the cytosol, nuclear entry, transcription and translation. Importantly, DNA must be released from the nanoparticles before transcription.

Poly(β-amino esters) (PBAEs) [10–13] are promising agents for non-viral gene delivery due to their (1) large potential for structural diversity, (2) ability to condense DNA into small and stable nanoparticles, (3) ability to buffer the endosome and facilitate endosomal escape, (4) biodegradability via hydrolytic cleavage of ester groups, (5) low cytotoxicity compared with some other cationic polymers, and (6) relatively high efficacy *in vitro* and *in vivo*. The best PBAEs are linear, synthesized at an amine/acrylate ratio of 1.2:1, and have a molecular weight of ~10 kDa [13,14].

Steady state fluorescence measurements of ethidium bromide are widely used to characterize DNA binding by cationic polymers and lipids. Due to the overlapping and broad spectra of free and DNA bound ethidium bromide, this method cannot be used in quantitative manner to resolve the binding constants of DNA and cationic polymers. Previously [15] we demonstrated that the nanoparticle formation and DNA–polymer binding constants and possible multiple states of binding can be determined with time-resolved fluorescence using ethidium bromide (ETI) as the fluorescent probe. Because fluorescence lifetimes and their proportions instead of intensity are used to analyze the state of the system, the method is not hampered by scattering and thus allowing more quantitative analysis than steady state measurements. The method revealed DNA complexation differences between an efficient transfection agent (polyethylenimine; PEI) and poor transfection agent (poly-L-lysine;

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doi:10.1016/j.jconrel.2011.06.016

PLL). For these polymers both the nature and the density of the amine groups taking part in the complexation of DNA are different: PEI contains primary, secondary and tertiary amines, whereas PLL includes only primary amines.

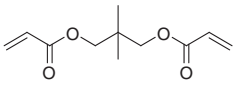
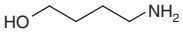
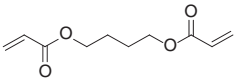
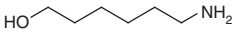
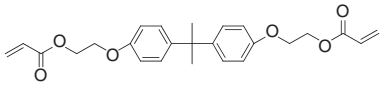
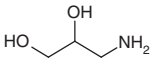
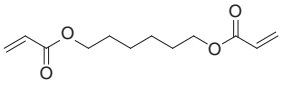
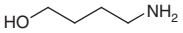
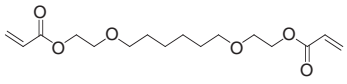
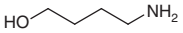
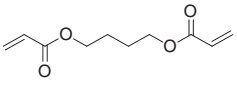
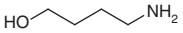
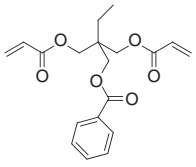
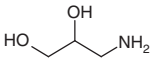
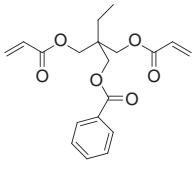
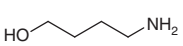
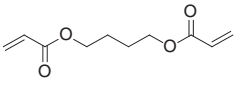
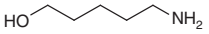
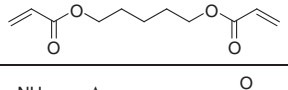
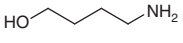
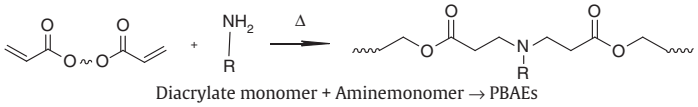
The purpose of this study was to extend the detailed DNA binding constant analyses to polymers with only tertiary amines. From a large library of PBAEs [13] ten polymers with different transfection efficacies were chosen for this purpose. We determined the DNA–complexation behavior of different PBAEs over a wide amine to phosphate (N/P) range, from 1 to 100, to reveal the complexation efficiency and mechanism, and to determine the binding constants for the studied PBAEs. In addition, we investigated a possible correlation between fluorescence parameters and transfection efficacy of PBAEs.

2. Materials and methods

2.1. Materials

Plasmid DNA (pCMVβ) that encodes β-galactosidase reporter gene was produced in *Escherichia coli*, and isolated and purified using a Qiagen Plasmid Giga kit (Qiagen, Germany). Ten different poly(β-amino esters) (PBAEs) (Table 1) with average molecular weights ranging from 8 to 28 kDa were synthesized by the conjugate addition of amine monomers (numbers) to diacrylate monomers (letters) solvent free at 95 °C or in DMSO at 60 °C [12]. The reaction proceeds in one step without the production of any byproducts. The PBAEs were then dissolved in DMSO to 100 mg ml^{-1} concentration and were further diluted into 50 mM MES-HEPES buffer (pH 7.4) to a final concentration of 6 mg ml^{-1}.

Table 1
Structures of the diacrylate and amine monomers of the studied PBAEs, their average molecular weights (MW) and amine:acrylate ratios (Am:Ac).

Polymer	MW (Da)	Am/Ac	Diacrylate monomer	Amine monomer
F28	16100	1.025		
C36	21200	1.2		
D24	9542	1.05		
E28	14300	1.1		
U28	15600	1.1		
C28	27900	1.05		
AA24	8058	1.3		
AA28	20900	1.1		
C32	18100	1.2		
JJ28	16800	1.1		
Synthesis of PBAEs			 Diacrylate monomer + Aminemonomer → PBAEs	