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DNA–polycation complexes Effect of polycation structure on physico-chemical and biological properties

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Abstract

The purpose of the study was to investigate the influence of cationic polymer structure on the formation of DNA–polycation complexes and their transfection activity. Primary, tertiary, and quaternary polyamines with molecular masses ranging from 8000 to 200,000 were investigated. DNA–cationic polymer interaction was characterized by low gradient viscometry, dynamic light scattering, circular dichroism, UV spectrometry, flow birefringence, DNA electrophoresis, and electron microscopy. Transfection activity of the complexes was evaluated by the expression of reporter gene (β-galactosidase) and using synthetic FITC-labelled oligonucleotides. Complex formation was found to be dependent on the structure and molecular weight of the polymer and the ionic strength of the solution. Secondary DNA structure in complexes was not disrupted, and DNA was protected from protonation. Cell lines of different origin were used for testing of transfection activity of the complexes. The sensitivity of the cells to transfection was established to be highly dependent on the cell line. DNA–polycation complexes are non-toxic according to MTT. Polyallylamine, and polydimethylaminoethylmethacrylate were found to be the most promising polycations for gene delivery. Transfection efficacy of their complexes with DNA to T-98G cells reaches up to 90–100%. It was found that optimal molecular mass of polydimethylaminoethylmethacrylate is in the range of 8000–50,000 Da.

Keywords: Polycation–DNA complexes; Polyallylamine; Polydimethylaminoethylmethacrylate; Transfection activity

1. Introduction

The gene transfer into intact cells is used now for the solution of various problems in biology and

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medicine. Synthetic polycations are known to be perspective agents for the non-viral gene delivery. They exhibit many advantages over other DNA transporting agents because of their ease of production, non-immunogenicity, low risk of side effects, simplicity of DNA–polymer complex formation. Polycations and DNA form compact interpolyelectrolyte complexes (IPEC) in a solution by electrostatic bonds between negatively charged DNA phosphate groups and positively charged groups of polycations (Kabanov and Kabanov, 1995). To form IPEC, poly(L-lysine), polyethyleneimine, polyvinylpyridines, cationic polymethacrylates, polyamidoamines, and dendrimers based on them, block and graftcopolymers of cationic and neutral hydrophilic monomers are used (Thomas and Klibanov, 2003; De Smedt et al., 2000; Eldred et al., 2005; Akinc et al., 2005; Dubruel et al., 2004; Reschel et al., 2002; Howard et al., 2004; Bos et al., 2004; Wolfert et al., 1999; Zhou and Li, 2004). Polycation molecules can include specific ligands providing targeted bonding to the cell membranes for penetration into the cell by the receptor-mediating pathway.

The efficiency of transfection can be increased by optimizing the polycation structure. However, for this purpose it is necessary to understand clearly the molecular basis of IPEC formation, i.e. the information is needed about conformational changes in secondary and tertiary DNA structures as well as about polycation behaviour during IPEC formation. Investigations of DNA binding to polycations of different structure are therefore needed at various conditions (temperature, ionic strength, pH).

In this work the effect of polycation structure, its molecular mass and charge density on DNA–polymer binding in water solution as well as on IPEC transfection activity were studied by the complex of physical and biological methods.

2. Materials and methods

2.1. Cationic polymers

Fig. 1 shows the structures of polycations investigated in this work.

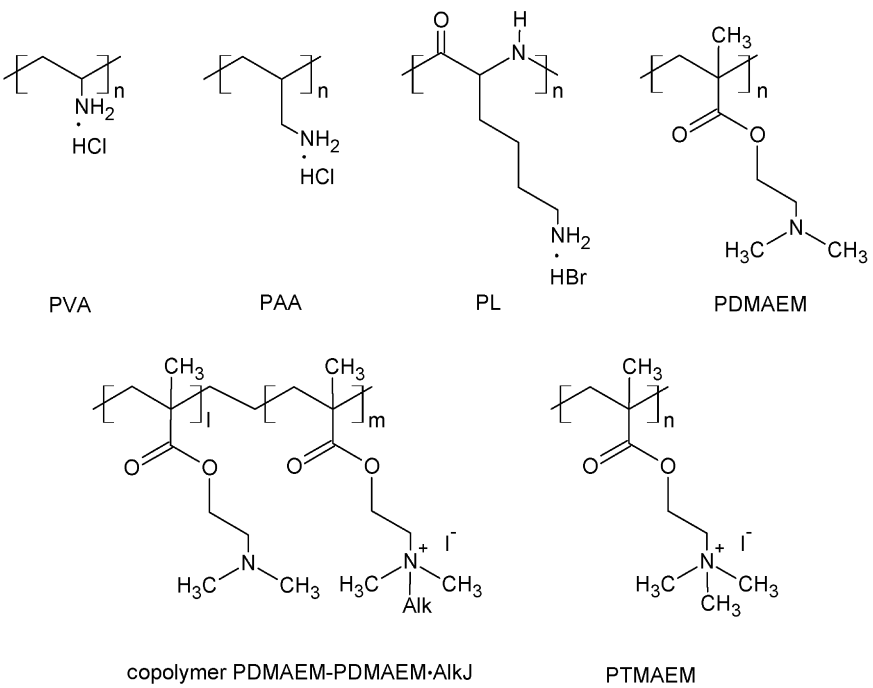

Fig. 1. Structure of polycations. (a) PVA (15,000), (b) PAA (8000, 12,000, 25,000), (c) PDMAEM (9000, 20,000, 30,000, 40,000, 50,000, 80,000, 150,000), (d) copolymer DMAEM–DMAEM·AlkI (Alk = C_2H_5, C_4H_9, C_8H_{17}; 30,000 or 40,000), and (e) PTMAEM (200,000).