STABILITY OF POLYCATION-DNA COMPLEXES: COMPARISON OF COMPUTER MODEL AND EXPERIMENTAL DATA

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Abstract
Polycations containing primary and tertiary amino and quaternary ammonium groups formed stable complexes with DNA only in pure water. Increasing concentration of sodium chloride in the saline solution resulted in dissociation, decomposition of the polyplexes and release of incorporated DNA. Stability of the complexes decreased depending on polycation substituent as follows: primary amine > secondary amine > tertiary amine > quaternary ammonium group. Results of computer modeling of the interaction of the oligocations with a model DNA chain were in good agreement with the experimental data.

Introduction

Polycation-DNA complexes (polyplexes) have been designed as potential vectors for in vivo gene delivery. Methods and conditions used for preparation of polyplexes, chemical structure of the polycations and their molecular weight are the most important factors influencing stability of the complexes in aqueous solutions, DNA-plasmid release and efficiency of gene transfection. One of the tasks of gene therapy is successful delivery of genes to the target cell and production of a therapeutic protein by the cell. However, a major obstacle in exploiting the full potential of gene therapy is the lack of a safe and efficient gene delivery system. Non-viral gene delivery systems based on complexes of cationic polymers with DNA or specific plasmids offer a promising approach to the solution of safe and effective gene delivery. For systemic delivery, the vector and its components should be non-toxic, non-immunogenic, stable in the blood circulation and small enough for effective extravasation. Efficient gene delivery system should enter the cell via receptor-mediated endocytosis, avoid degradation of DNA by lysosomal enzymes, ensure cytoplasmic mobility, nuclear trafficking and finally unpackaging in the cell nucleus [1]. In our previous papers we described preparation and study of properties of a variety of polycation-DNA complexes dealing with particular problems of their use for gene delivery, e.g. self-assembly and complex formation [2-4], stability of the complexes [5, 6] or their targeting [7, 8].

The aim of this work is an attempt to contribute to the elucidation of the influence of the polycation structure on interpolyelectrolyte interactions in the complexes of plasmid DNA with various polycations, proposed as nonviral vectors for the delivery of genes into the nucleus of the target cells and verify possibilities of computer modeling in design and prognostics of properties of the complexes.

Computer modeling of the self-assembly of a plasmid DNA double helix with a synthetic polycations is the first step of our attempt to describe the system based on the polyelectrolyte complexes between natural polyanions (DNA) and synthetic polycations. Gene delivery vectors of this type are assumed to protect DNA during the transport in body fluids and after extravasation in the cytoplasm of the target cell, and to release it in the nucleus to evoke the production of a therapeutic protein [9]. The starting model was based on results of our previous physico-chemical study of stability and transfection activity of DNA complexes [10]. In this study, the polycations used for self-assembly of polyplexes included cationic polymers and copolymers containing primary and tertiary amino and quaternary ammonium groups. It was found that the size of the complexes was small enough to ensure effective extravasation, it increased with increasing pH of the buffer and increasing temperature in the complex formation [10]. By decreasing the molecular weight of the respective polycation the complexes became smaller but, at the same time, less stable to complex dissociation and coagulation in aqueous solutions. DNA complexes with polycations containing primary amino groups showed the best stability in saline solutions with the stability of poly(lysine) complexes being superior to that prepared with methacryloylated aminoamides. Lower stability was found for complexes formed with tertiary amines and the least resistant to destruction and DNA release were complexes prepared with polymers containing quaternary ammonium salts. In transfection experiments, the highest transfection activity was found using complexes prepared from primary amino group-containing polycations and complexes with quaternary ammonium groups provided the lowest transfection activity.

Computional approach

Binding of the CH_2-R group to the phosphate. To select a polycation of sufficient energy of binding to the DNA phosphate groups, three types of possible monomers containing the CH_2-R group were tested (cf. Table 1), where R is primary, secondary and tertiary amino and quaternary ammonium groups. Dimethyl phosphate in its most stable *gauche*, *gauche* conformation [11, 12], was used as a model representing negatively charged DNA phosphate groups. To estimate the binding energy, quantum mechani-

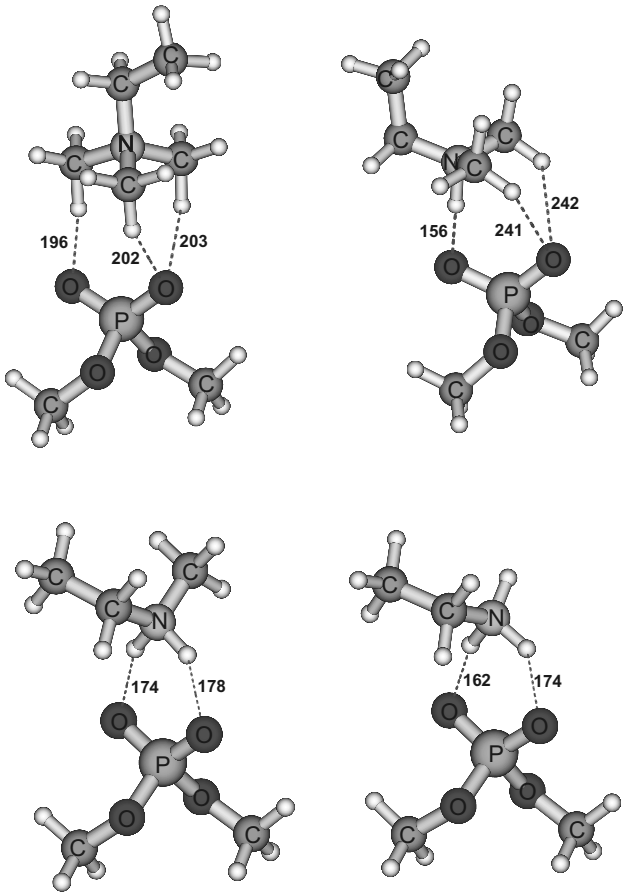

Figure1. MP2/6-31G* optimized model structures of complexes of CH_3CH_2-R with the phosphate group. Distances are in pm.

cal (Gaussian 98) [13] and molecular mechanical (Discover 2000) [14] calculations were performed. Quantum mechanical calculations were carried using the 6-31G* basis set which includes polarization functions on all atoms except hydrogens. Geometries have been fully optimized for the smallest systems at the second-order Moller Plesset (MP2) level of theory and for the remaining systems at the density functional theory (DFT) level using the B3LYP functional [15]. In accord with the experimental data [10], the lysine with primary amino group (Figure 1) showed the highest binding energy and with quaternary ammonium group the lowest binding energy. Similar results were obtained from the molecular mechanics (525 and 361 kJ/mol with primary amino and quaternary ammonium group, respectively). Calculations were also performed using suite of Biosym/MSI [14] programs, particularly the InsightII and the Discover programs. The cff91 force field with the dielectric constant =1 was used in all molecular mechanics calculations.

L-lysine oligocation. Positively charged oligo(L-lysine) was chosen as a proper candidate [16] to form a polycation in a complex with B-DNA under physiological conditions (pK_a 10.80) [17]. To show an optimal geometry of the positively charged oligo(L-lysine) fragment, molecular modeling of a free dodecamer of the fragment mentioned was performed. In potential energy minimization, in contrast to the uncharged oligo(L-lysine), the positively charged oligo(L-lysine) converged to a helix of similar conformation independently of the starting model (-strand, -helix, -helix, 3_{10}-helix). The rise per amino acid residue was always higher than 300 pm and the helical twist more than 100 in dependence on the starting model. The helix conformation shows the potential energy per one residue lower by 544 kJ/mol than the -strand conformation.

B-DNA fragment. The eleven-base-pair B-DNA duplex of 5'-(ATATATATATA)-3' was arbitrarily chosen from the standard structures of InsightII program [14] to simulate part of the plasmid. It can be shown that positions of the B-DNA-OPO_3- groups are properly oriented to meet the positively charged oligo(L-lysine) -NH_3^+ groups of the interacting oligocation. The degree of the charge compensation N/P close to unity supports the model where the flexible positively charged oligo(L-lysine) b-strand embeds into the major groove of the B-DNA.

Docking of oligo(L-lysine) in the B-DNA. A system of eleven-base-pair B-DNA rigid duplex fragment and positively charged flexible hexamer of L-lysine was introduced into the final attempt of docking procedure simulation. The aim of this attempt was to draw a qualitative model only. Suite of Biosym/MSI [14] programs was utilized under the same conditions as mentioned previously. A simplified approach was chosen in all calculations. Both molecules were assumed to be in vacuo in the absence of other molecules (water, ions, etc.). Generic distances between six -NH_3^+ and -OPO_3^- groups were introduced with an upper bound of 500 pm. Below the 500 pm limit, the additional attraction was set to zero. The steepest descent (22,000 iterations) and conjugate gradients (10,000 iterations) of potential energy minimization were automatically stopped when the gradient of potential energy was lower than 0.42 kJ/mol.nm.

In our model we assume that water molecules, small cations and anions are pushed away of the B-DNA surface and, therefore, they do not play a significant role in shielding the phosphate groups. Our proposed model (Fig. 2) shows the positively charged oligo(L-lysine) flat strand embedded in the major groove of the B-DNA molecule. The -NH_3^+ groups are pointing to the -OPO_3^- groups with a rather long average distance between oxygen and nitrogen atoms of 381(5) pm. For a reliable quantitative model, a complex calculation, including flexible and longer B-DNA duplex, would be necessary.

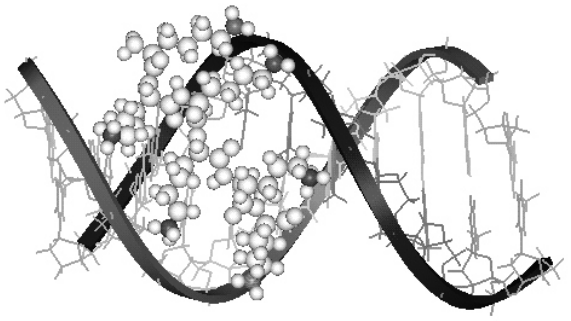

Figure 2. B-DNA - positively charged oligo(L-lysine) interaction. Hexamer of the positively charged oligo(L-lysine), balls of 0.5 vdW radius are embedded in the major groove of B-DNA (spaghetti model).

Discussion

Three types of monomers mentioned in Table 1 show the same trend in binding energies. This supports the idea that the ligand structure (containing a double bond, single bond, short fragment only) has no decisive influence on the binding process. All three structures modeling various cationic monomer units incorporated in the polycation structure demonstrate the highest energy of interaction with phosphate group of the DNA chain for methacryloyl-based units containing primary amino group. The binding energy is decreasing in the order: primary amine > secondary amine > tertiary amine > quaternary ammonium group. This finding is in a very good agreement with our experimental data [10] describing the stability of polycation/DNA complexes in saline solutions. Here, the NaCl concentrations at which 50 % of DNA is released from the complex (c_s^{50}) was used as the dissociation stability characteristics. In agreement with the calculated binding energy, the experimental data show the highest stability for DNA complexes formed by poly(L-lysine) and poly(AEMA) (primary amines, $c_s^{50} \sim 1.3$ mol/l), lower for complexes containing poly(DMAEMA) (secondary amine, $c_s^{50} \sim 0.7$ mol/l) and the lowest for poly(TMAEM) (quaternary ammonium group, $c_s^{50} \sim 0.5$ mol/l) [10]. The alphameric symbols of the compounds were chosen in accord with the definition given in previous paper [10]. For their chemical structure and explanation of the symbols, see Table 1. The agreement between theoretical and experimental data verifies the use of approximations in model calculations and, at the same time, it demonstrates the potential of a computer modeling in designing polyplexes as vectors for efficient gene delivery.

Conclusions

Despite the rather rough approximation used in this modeling, a reasonably simple model of B-DNA - positively charged poly(L-lysine) complex was designed, which appears to be in agreement with previous physicochemical experimental results. Also the binding energy calculated for interaction of various oligocations with the phosphate groups in model DNA chain is in good agreement with experimental data obtained earlier, demonstrating a drop in the polycation-DNA complex stability in dependence on the polycation structure in the following order: primary amines > secondary amines > tertiary amines > quaternary ammonium group. The agreement of calculated and experimental data verified the feasibility of computer modeling and calculation in designing polycation-based gene delivery systems.

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Table 1. Binding energy (kJ/mol). The alphameric symbols of the compounds were chosen in accord with the definition given in [10]: AEMA *N*-(2-aminoethyl)metacrylamide, AEPA *N*-(2-aminoethyl)pivalamide, AE aminoethane, M methyl, DM dimethyl, TM trimethyl.

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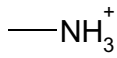
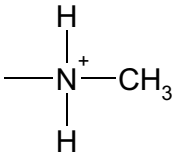
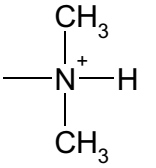
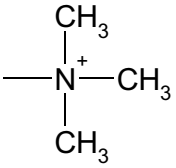
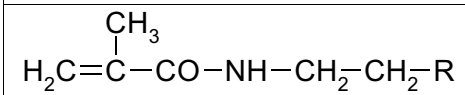
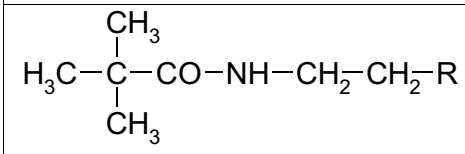
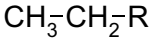
Model compound	 primary amino group	 secondary amino group	 tertiary amino group	 quaternary ammonium group
 B3LYP/6-31G*	AEMA 464	MAEMA 455	DMAEMA 433	TMAEMA 390
 B3LYP/6-31G*	AEPA 453	MAEPA 448	DMAEPA 423	TMAEPA 390
 MP2/6-31G*	AE 531	MAE 515	DMAE 501	TMAE 409