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Cationic Oligopeptides Modified with Lipophilic Fragments: Use for DNA Delivery to Cells

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Abstract—Cationic oligopeptides, including the amphipathic α-helical peptides, are applied to the targeted delivery of DNA to eukaryotic cells due to their DNA-compacting properties and the ability to destabilize the cell lipid bilayer in some cases. We synthesized the peptides differing in the number and location of residues of decanoic acid covalently attached to Lys residues in order to combine the DNA-binding and the membrane activities in a single molecule. We chose peptide structures that assisted in the formation of α helices. The DNA-binding ability of the peptides and the membrane activity of their complexes with DNA were shown to depend on the structure. The study of erythrocyte hemolysis by complexes with DNA of the pCMV *LacZ* plasmid and the peculiarities of transfection of these complexes revealed a correlation between the hemolytic activity and the expression level of the *lacZ* gene in cells.

Key words: cationic α-helical oligopeptides, hemolysis, nonviral DNA carriers, transduction, transfection

INTRODUCTION

The search for effective methods of systemic delivery of genes to eukaryotic cells is one of the most important areas of development of gene therapy [1–4].[2] Three approaches are mainly used for the insertion of marker or "therapeutic" genes into cDNA: (1) application of recombinant viral vectors; (2) physical transfection methods (such as electroporation, DNA injections, "gene gun" method, and others); and (3) the application of nonviral systems for the DNA delivery to cells [5, 6]. The carriers on the basis of recombinant viruses (e.g., adenovirus ADV), retroviruses (RV), and herpes simplex type 1 virus (HSV-1) have a number of advantages over the other delivery methods due to their high specificity to certain types of tissues and their ability to be effectively transfected. However, this method has some serious drawbacks: potential pathogenicity, possibility of a high immune response, and a limited quantity of DNA that can be packed into small particles. Therefore, a search for nonviral vector systems devoid of these drawbacks and displaying some advantages of viral carriers is an urgent problem. The modeling of the virus penetration into cells is one of the approaches to the search for nonviral transfection systems. The choice of a model depends on its ability to form compact particles of the transforming DNA or RNA, to be the specifically bound by the cell surface, to penetrate trough cell membrane structures, and to have a distinct signal of nuclear localization for the active transport into nucleus [7–10].

The membrane-active fragments of viral proteins often are α-helical and amphipatic [11, 12]. In this study, we describe the synthesis of a number of peptides with various contents and locations of hydrophobic amino acid residues and Lys residues, including the Lys residues acylated with the residues of decanoic acid. These peptides combined the complex-forming and membrane-active properties in a single molecule. We found that the hemolytic activity and the helicity degree of the synthesized peptides depended on their decreased hydrophobicity. We also demonstrated the ability of the peptides to form the complexes with DNA, to protect DNA from the cleavage by nucleases, and to facilitate the DNA transport into cell.

RESULTS AND DISCUSSION

The introduction of alien genes into cell nucleus is one of the important problems of gene therapy. A high efficiency of the viral gene delivery is still not achieved despite all the efforts in the field of search for the viral systems transporting gene material into cells. An artificial DNA carrier should meet a number of demands, in particular, it should be able to compact DNA and transfer it trough cell membranes. This problem is often overcome by the use of liposomes (lipoplex) [13]. The

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[2] Abbreviations: Acm, acetamidomethyl; Ahx, residue of ε-aminocaproic acid; DIC, diisopropylcarbodiimide; TFA, trifluoroacetic acid; and TFE, trifluoroethanol.

Table 1. Structure of the synthesized peptides synthesized

Peptide	Structure*	M_{exp}
FP	C(Acm)–Ahx–YKAKKKKKKKWK–NH_2	1877.1
FP1	C(Acm)–[K(C10)]–Ahx–YKAKKKKKKKWK–NH_2	2161.6
FP4	C(Acm)–Ahx–WK[K(C10)]KK[K(C10)]KKK[K(C10)]KYK[K(C10)]KK–NH_2	3063.0

* K(C10) is N^{ε}-decanoyllysined.

use of membrane-active compacting peptides can significantly increase the transduction efficiency. Such peptides very frequently have an amphipatic α-helical structure, with one of the helix side being hydrophilic and another being hydrophobic [14–16]. Such structures are formed by the alternating hydrophilic and hydrophobic amino acid residues (positions $i \longrightarrow i + 3/4$) [17–19]. One of the examples of such peptides is JTS-1 (GLFEALLELLESLWELLLEA) whose introduction into the complex of the K-8 peptide ($YKAK_8WK$) with DNA sharply increases (by 6000 times) the expression level of luciferase gene in the HepG2 cells [6, 20]. However, JTS itself cannot bind DNA owing to the absence of positively charged groups in its molecule. In this study, we synthesized a number of peptides with various contents and locations of unnatural hydrophobic residues (Table 1), in order to find the structure that could form complexes with DNA and, simultaneously, increase the interaction of such carrier with the cell membrane [21]. Also, we plan further to modify the peptides through sulfhydryl group and, therefore, attached the *N*-terminal Cys residues linked to the basic sequence via an Ahx residue.

Ohmori *et al.* demonstrated by the example of mastoparan [17] that the introduction of one hydrophobic residue into a peptide chain did not prevent interaction of the peptide with nucleic acid and can also contribute to the appearance of the peptide ability to form a compact structure. The introduction of the capric (decanoic) acid residue proved to be optimal in this case. Further increase in the hydrophobic radical length did not lead to a significant increase in the binding ability. In addition, one can expect a decrease in the solubility of such a compound in water along with a further increase in the length of hydrophobic radical. Therefore, it was decanoic acid that we here chose for the modification of ε-amino groups of Lys residues in cationic peptides.

The purity of the peptides synthesized was determined by HPLC, and their structures were confirmed by mass spectrometry (see Figs. 1, 2).

The study of the peptide structures by CD. The structural characteristics of the peptides synthesized were determined from their CD spectra under various conditions (Fig. 3). One can see from the results (Table 2) that the helicity of the peptides increased with the increase in their hydrophobicity; however, the structure became unordered in acidic medium due to repulsion of positive charges of the ε-amino groups of Lys residues. On the other hand, the neutralization of negative charges of Glu residues of JTS-1 in acidic medium led to an increase in the content of α-helices. The maximum helicity of the peptides was observed in 80% trifluoroethanol, because its dielectric properties are similar to cell membranes and it is often used for the conformational studies of peptides in hydrophobic environment. Thus, we could expect a significant membrane activity for the peptides modified with the residues of decanoic acid owing to a high content of α-helices in them and their hydrophobicity.

Hemolytic activity of the peptides. Endosomolytic capacity (i.e., the ability to penetrate through the endosome membranes into the cell cytoplasm) is an important factor in the penetration of DNA with the carrier into a cell. Erythrocyte membranes were chosen as a model of biological membranes. The hemolytic activity depended on the peptide hydrophobicity and the medium pH (Fig. 4). The hemolytic activity increased along with the increase in hydrophobicity: FP4 ≈ JTS-1 > FP1 > FP. The FP peptide had no effect on the erythrocyte membranes. The FP4 and the JTS-1 peptides exerted the maximum activity that was exhibited at the pH values causing no aggregation due to the decrease in the number of charges on the Glu and Lys residues. Unlike the case of FP4, the hemolytic activity of JTS-1 increased with the decrease in its concentration and, then, sharply decreased [6]. At the same time, the value of medium pH did not affect the FP1 activity. Thus, a correlation between the hemolytic activity and the content of α-helical conformation was observed in the peptides. The peptides adopt this conformation in hydrophobic environment, probably including the erythrocyte membrane. One can expect that the hydrophobic peptides would more effectively interact with cell

Table 2. Percentage of the α-helical regions in the peptide structure in various media

Peptide	0.01 N NaOH	0.01 N HCl	80% TFE
FP	8	Unordered	25
FP1	25	Unordered	70
FP4	27	Unordered	63
JTS-1	15	45	70

membranes and intracellular structures than the peptide not modified with decanoic acid residues. The pH-dependent membrane activity of the FP4 peptide that was similar to the membrane activity of JTS-1 should probably facilitate the release of the complex from the cell endosomes.

The formation of DNA–peptide complexes. The ability of the peptides to the binding to DNA was examined by gel electrophoresis of DNA–peptide complexes with different molar ratio of the components (method of gel retardation) [22]. The electrophoretic mobility of the complexes gradually decreased with the increase in the peptide amount in complex (Fig. 5b). The complete retardation of the plasmid mobility took place even at the charge ratio 1 : 1 for DNA–FP peptide and 1 : 0.5 for DNA–FP1 peptide. In the case of FP1, an increase in the complex density was observed for along with the increase in the carrier content in complex. This fact was proved by the practically complete disappearance of fluorescence of the ethidium bromide bound to DNA in the gel wells. The complete DNA retardation proceeded at the charge ratio of DNA–FP4 of 1 : 0.7.

Protection of DNA within the complex with amphipatic peptides from DNase I. The complete protection of plasmid from the action of hydrolytic enzymes requires a higher content of the peptides synthesized in complex than that necessary for the formation of completely compact DNA conformation. This was proved by studying the stability of plasmid DNA within the DNA–FP, DNA–FP1, and DNA–FP4 complexes to the action of DNAase I (Fig. 6). The effective protection of the plasmid DNA by the FP carrier from the enzyme began with the charge ratio of 1 : 1.4. We can see in the gel the fragments of cleaved DNA along with the conformation forms characteristic of the intact plasmid at a charge ratio of 1 : 0.7, which suggested that the plasmid was partially cleaved at this charge ratio. A sufficiently effectively protection of DNA by the FP1 and FP4 peptides began starting from the ratio of 1 : 0.7.

The efficiency of cell transformation by the DNA–FP, DNA–FP1, and DNA–FP4 complexes. The cell transformation by the complexes of peptides FP, FP1, and FP4 with the marker *LacZ* gene (plasmid pCMV*LacZ*) was studied in each case at the DNA–carrier ratios close to those corresponding to the maximum DNA protection from the action of DNase I (Fig. 7). We found that the transformation efficiency that was provided by the FP peptide–carrier was less than 0.01% and was reliably the same as that of the native DNA. The maximum transformation efficiency when using the FP1 peptide was achieved at the DNA–carrier charge ratio of 1 : 2.8 and was approximately 0.34 ± 0.05% of the cells. The most effective DNA–FP4 charge ratio was 1 : 1.4; it provided the expression of 3.61 ± 0.7% of the cells. Thus, the introduction of four residues of decanoic acid significantly increased the expression level of the plasmid. This result can be explained by a rather high rate of release of the complex from the cell endosomes due to the membrane activity by the peptide itself.

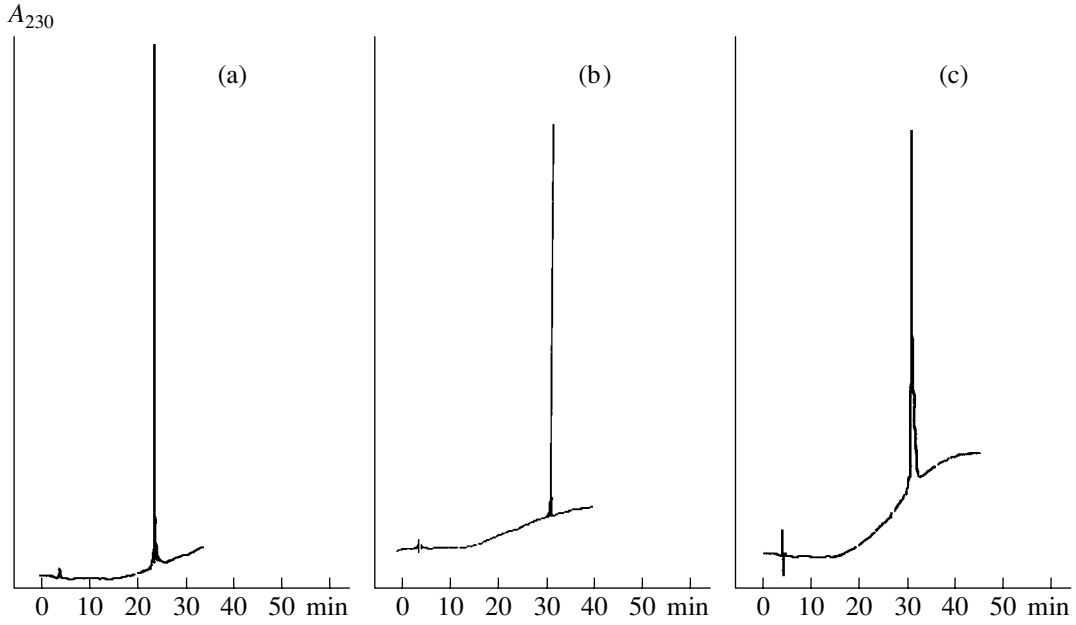

Fig. 1. Analytical HPLC of the (a) FP, (b) FP1, and (c) FP4 synthesized peptides on the Nucleosil C18 column (4.6 × 150 mm) eluted with a gradient of acetonitrile in 0.1% TFA [(a, b) from 10 to 40% and (c) from 30 to 60% of acetonitrile within for 25 min] at a flow rate of 1 ml/min; detection at 230 nm.

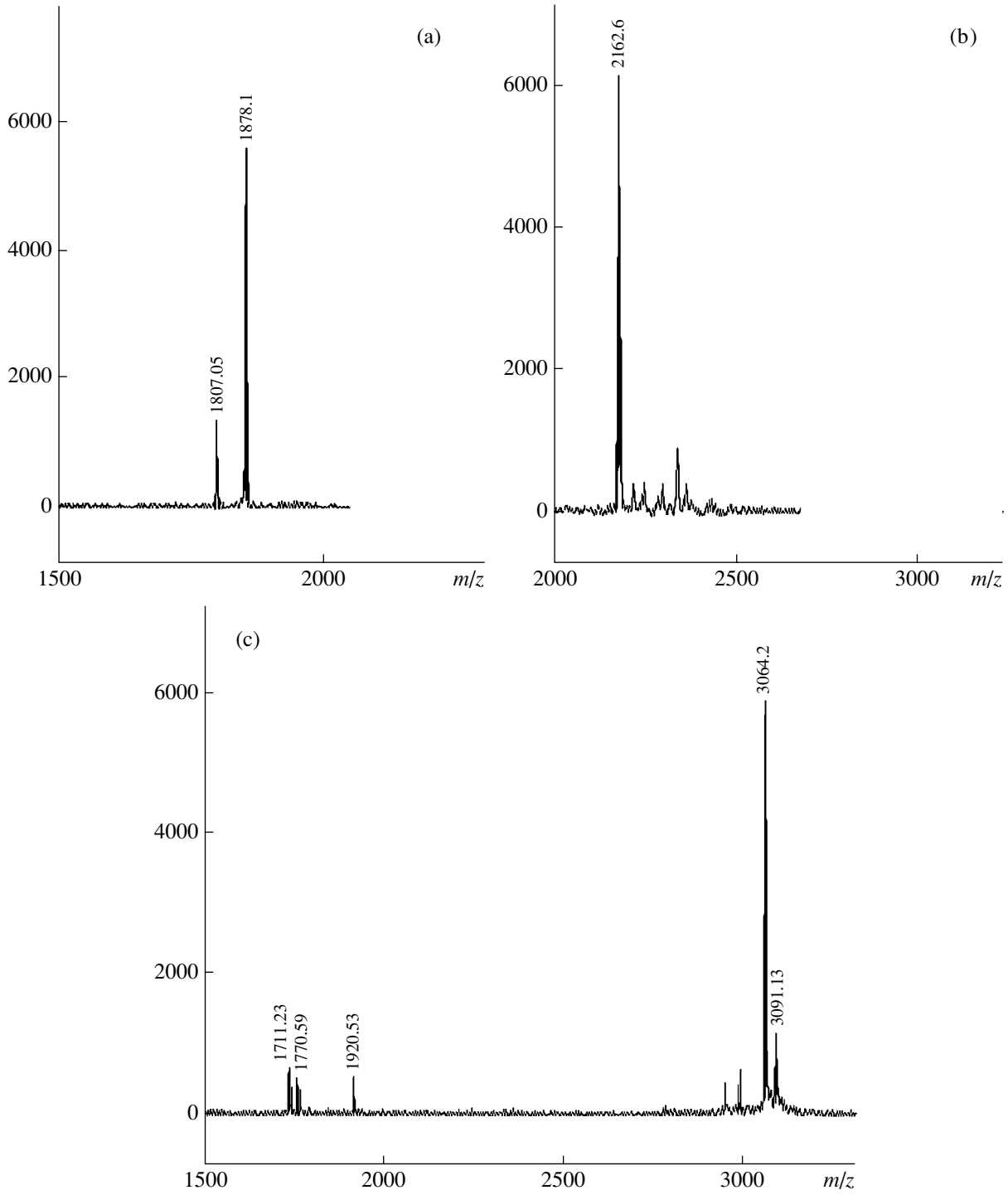

Fig. 2. MALDI TOF mass spectra of the (a) FP, (b) FP1, and (c) FP4 peptides in water (pH 6.4).

The endosomolytic properties of the FP1 and FP4 peptides were investigated after a number of transformations of the cells *in vitro* by the DNA–peptide complexes in the presence of chloroquine that disturbs the process of endosome acidification and, by such a way, prevents the action of hydrolytic enzymes. The treatment of cells by chloroquine provides the most complete exhibition of the intrinsic membrane-active properties of the carrier. These experiments demonstrated that the presence of chloroquine increased the efficiency of transfection by the DNA–FP1 complex from $0.08 \pm 0.02\%$ to $5.15 \pm 0.31\%$. On the other hand, chlo-

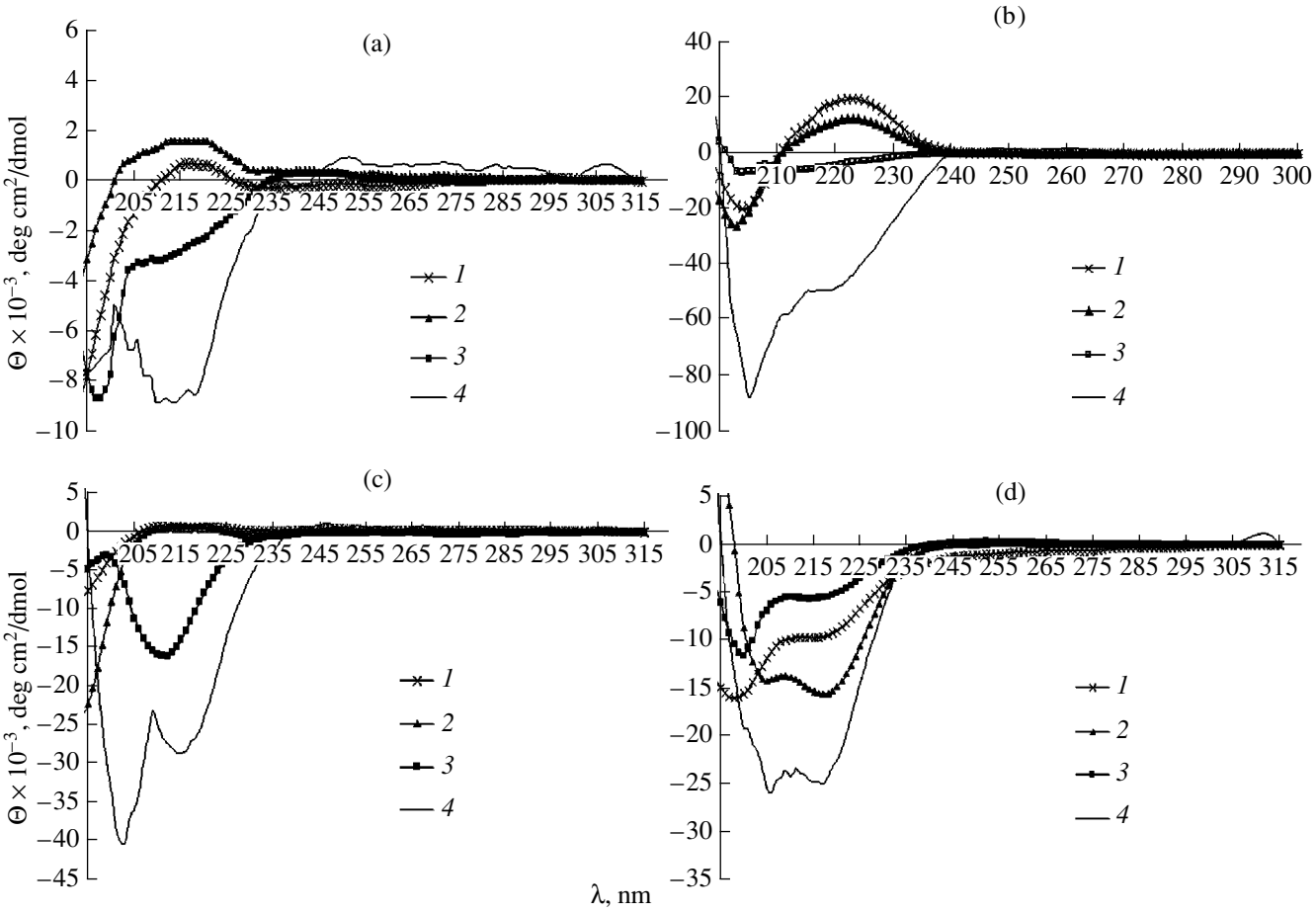

Fig. 3. CD spectra of the peptides (a) FP, (b) FP1, (c) FP4, and (d) JTS-1 in (*1*) water (pH 6.4), (*2*) HCl (pH 3.0), (*3*) NaOH (pH 10.0), and (*4*) 80% TFE.

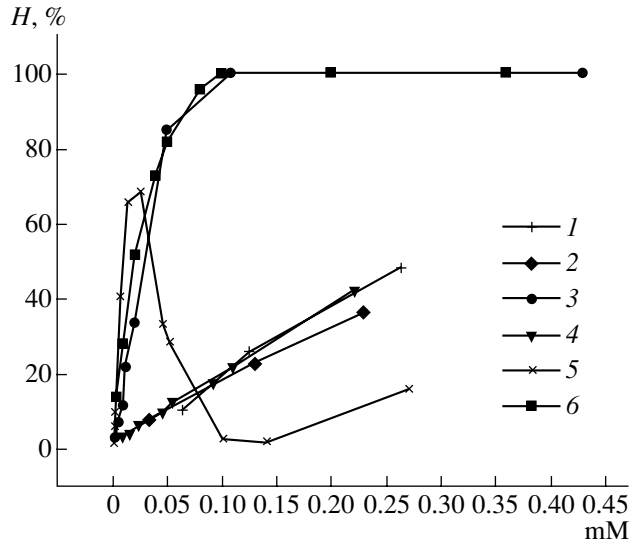

Fig. 4. Hemolytic activity of the peptides (*1*, *2*) FP1, (*3*, *4*) FP4, and (*5*, *6*) JTS-1 at (*1*, *3*, *5*) pH 5.0 and (*2*, *4*, *6*) pH 7.8.

roquine exerted little effect on the efficiency of transfection by the DNA–FP4 complex: the content of transfected cells remained practically the same.

On the basis of these results, we hypothesized that the carrier FP4 peptide itself had endosomolytic properties, which are much more pronounced than those of the FP1 peptide. This result agrees well with the experiments of erythrocyte hemolysis. The similarity of hemolytic properties of peptides FP4 and JTS-1 suggests that their endosomolytic properties are similar. However, the FP4 peptide can compact DNA and protect it from the action of hydrolytic enzymes present in cell endosomes and lysosomes, which differs it from the JTS-1 peptide.

CONCLUSIONS

We synthesized and studied a number of peptides with different content and location of the residues of hydrophobic amino acids and lysine, including the Lys residues modified by decanoic acid, and demonstrated that the ability of the studied peptides to transfect cells

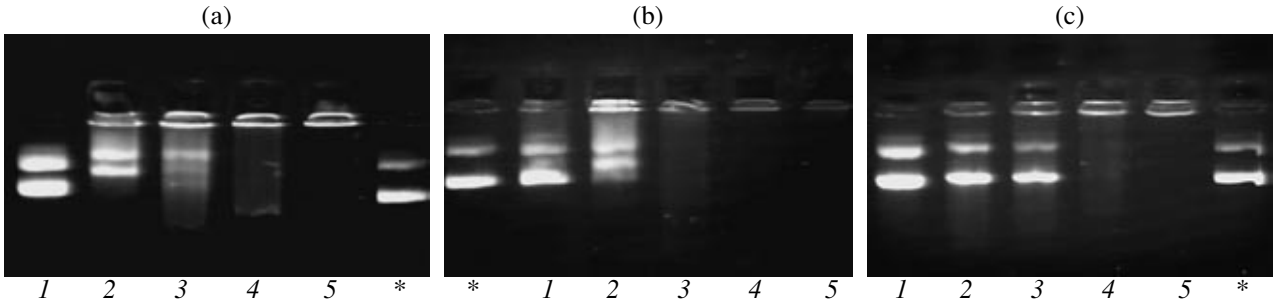

Fig. 5. Evaluation of the efficiency of binding of the pCMV*lacZ* plasmid with the peptides by the electrophoresis of DNA complexes with (a) FP, (b) FP1, and (c) FP4 in 0.8% agarose gel containing ethidium bromide (0.5 μg/ml) at the DNA–peptide charge ratio of (*1*) 1 : 0.1, (*2*) 1 : 0.3, (*3*) 1 : 0.5, (*4*) 1 : 0.7, and (*5*) 1 : 1. The intact DNA is marked with an asterisk.

with DNA increased with the increase in the number of decanoic acid residues in these peptides. We consider the FP4 peptide to be currently the most promising compound that exhibits a pronounced endosomolytic activity among all the studied peptides. A further modification of the FP4 peptide (introduction of the signal of nuclear localization and the ligand for the directed transport to the desired cell types) should enhance its endosomolytic and transfection properties and could significantly increase the gene expression level.

EXPERIMENTAL

Derivatives of *L*-amino acids, DIC, TFA, trifluoromethanesulfonic acid, thioanisole, ethanedithiol, 1-hydroxybenzotriazole, (Fluka, Germany), 5-bromo-4-chloro-3-indolyl β-*D*-galactopyranoside (X-gal, Sigma, United States), DNase I (Promega, United States), chloroquine, proteinase K (Sigma, United States), and Escort TM (Promega, United States) were used in this study. The solvents were from OAO Vecton (St. Petersburg) and purified before use. The transfection experiments *in vitro* were carried out on cell cultures of the HeLa human epithelial carcinoma (Institute of Cytology, Russian Academy of Sciences, St. Petersburg). The cells were cultured on the RPMI 1640 medium (OOO Biolot, St. Petersburg), containing calf blood serum (OOO Biolot, St. Petersburg) and gentamycin (OAO Dal'khimfarm, Khabarovsk).

The pCMV*LacZ* plasmid DNA (kindly presented by Dr. B. Scholte from Erasmus University, Rotterdam) was used in the experiments on DNA compacting, DNA protection from enzymatic cleavage, and the plasmid transformation. The complex formation was studied by CD using DNA from calf thymus with molecular mass of 8.6 MDa (Serva, Germany).

Equipment. The peptides were synthesized on an NPS 4000 semi-automatic peptide synthesizer (Neosystem Laboratories, France) on a *p*-methylbenzhydrylamine resin (Neosystem Laboratories, France). The peptides were purified by the gel exclusion chromatography on a Bio-Gel P2 (BioRad Laboratories, United States) with the detection on an Uvicord S 2138 (Prague). The products were further purified by the reversed phase HPLC on a Waters 600E chromatograph (Waters, United States) equipped with a Vydac C-18 analytical column (4.6 × 150 mm, Supelco, United States) and Vydac C-18 preparative column (22 × 250 mm, Supelco, United States). The amino acid analysis was performed on an AAA T339 M analyzer (Microtechna, Prague, Czech Republic). TLC was carried out on Silufol plates (Kavalier, Czech Republic).

The MALDI TOF mass spectrometry was performed on a Voyager-DE spectrometer (United States) using α-cyano-4-hydroxycinnamic acid (State Technical University, St. Petersburg). CD spectra were recorded on a Mark V dichrograph (Gobin Ivan, France). Cells were counted in the transformation experiments using a light microscope (Karl Zeiss, Germany).

Peptide synthesis. N^{ε}-Decanoyl-N^{α}-*tert*-butyloxycarbonyllysine. A solution of *N*,*N*'-dicyclohexylcarbodiimide (2.3 g, 0.011 mol) in chloroform (15 ml) was added to a solution of decanoic acid (1.7 g, 0.01 mol) and pentafluorophenol (2 g, 0.011 mol) in chloroform (25 ml) at cooling with ice. After 3 h, acetic acid (several drops) was added to the reaction mixture, the precipitated *N*,*N*'-dicyclohexylurea was filtered off, and the filtrate was evaporated in a vacuum. Triethylamine (2 ml) and α-*tert*-butyloxycarbonyllysine (2.5 g, 0.01 mol) were added to the solution of pentafluorophenyl ester of decanoic acid in DMF (25 ml). After 24 h, the reaction mixture was diluted with 1 N sulfuric acid (200 ml), and the product was extracted with ethyl acetate. The ethyl acetate solution was two times washed with water, dried over anhydrous sodium sulfate, and evaporated to get the title product; yield 3.7 g; R_f 0.62 (85 : 10 : 5 chloroform–methanol–acetic acid) and 0.4 (1 : 3 petroleum ether–ethyl acetate).

Solid phase peptide synthesis was performed according to the Boc-strategy using *p*-methylbenzhydrylamine resin as a polymer support, DIC/HOBt as a coupling reagent, and trifluoroacetic acid as a deprotecting reagent. The following protocol was used when calculating for 1 g of resin with the capacity of

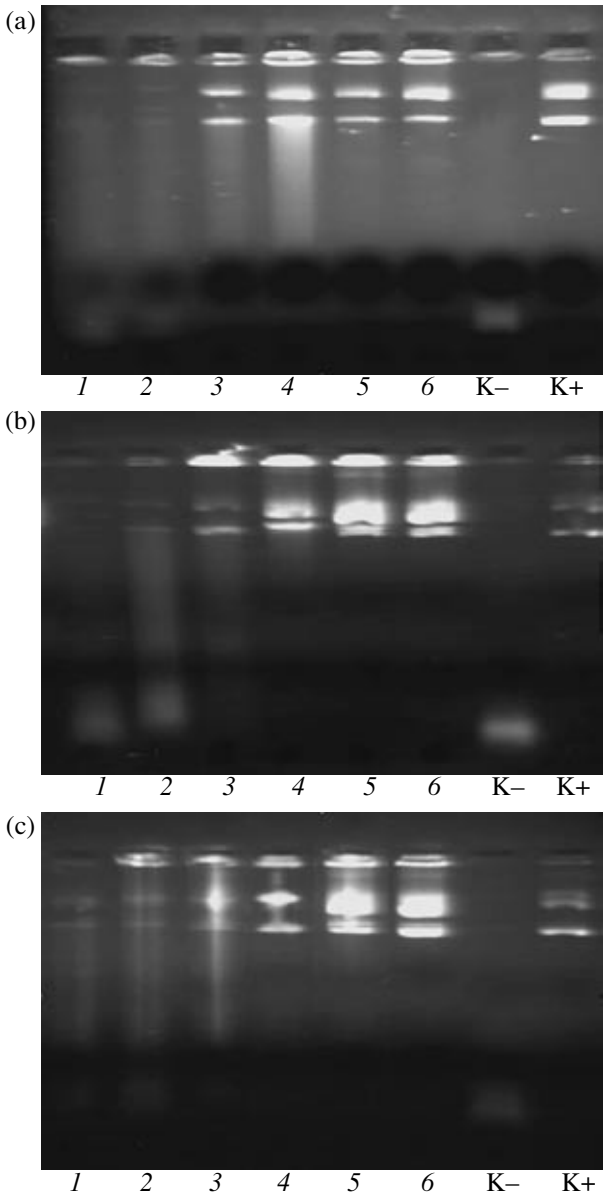

Fig. 6. The efficiency of protection of the plasmid DNA from DNAase I by (a) FP, (b) FP1, and (c) FP4 at the charge DNA–peptide ratio of (*1*) 1 : 0.2, (*2*) 1 : 0.4, (*3*) 1 : 0.5, (*4*) 1 : 0.7, (*5*) 1 : 1.4, and (*6*) 1 : 2.8 compared with (K–) the action of DNaase I without the peptides and with (K+) the intact DNA.

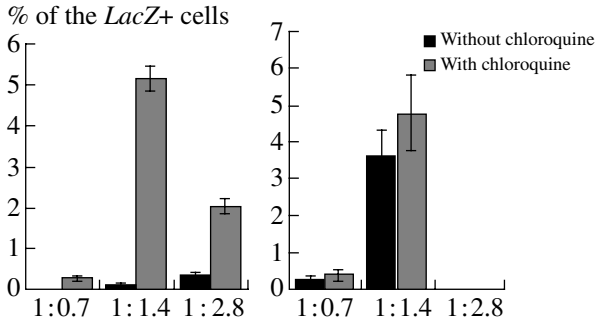

Fig. 7. The dependence of the efficiency of transfection of the HeLa cells on the charge ratios between DNA and the FP1 peptide and between the DNA and the FP4 peptide.

0.5 mmol/g: (1) twice deprotection with 65% TFA (2 × 1 min), and washing with dichloromethane (2 × 10 ml, 15 min); (2) washing with dichloromethane (3 × 10 ml) and DMF (2 × 10 ml); (3) deprotonation with 10% TEA for 1 min and washing with DMF (2 × 10 ml) for 2 min; (4) washing with DMF (2 × 10 ml); (5) coupling with the corresponding Boc-amino acid (1.5 mmol) using DIC (1.5 mmol) and 1 M solution of HOBt in DMF (1.5 ml); and (6) washing with DMF (2 × 10 ml) and dichloromethane (2 × 10 ml); monitoring with ninhydrin test. The protocol was repeated starting from stage 3 if the ninhydrin test was positive (incomplete coupling). The complete deprotection and the cleavage of the peptide from the polymer were achieved by the treatment with trifluoromethanesulfonic acid (1 ml) in TFA (10 ml) in the presence of thioanisole (1 ml) and ethanedithiol (0.5 ml) for 1 h at cooling with ice and for 1.5 h without cooling. The reaction mixture was diluted with ether (100 ml), and the precipitate was filtered. The resulting peptide was dissolved in TFA (30 ml), filtered for the removal of resin, and precipitated with anhydrous ether (200 ml). The nonadecapeptide JTS-1 was prepared according to the procedure [20]. The peptides were preliminarily purified by gel chromatography on a column (600 × 25 mm) with BioGel P-2 (Pharmacia, United States) eluted with 6% aqueous solution of acetic acid with detection at 226 nm.

The further purification of peptides was carried out by HPLC using 0.1% aqueous TFA as eluent A and mixtures of acetonitrile solution in eluent A as eluent B at a flow rate of 1 ml/min (for the analytical HPLC) and 10 ml/min (for the preparative HPLC). The purity of peptides was >95%. The structures of the synthesized peptides were confirmed by mass spectrometry and amino acid analysis.

Determination of hemolytic activity was done as described in [6]. Human erythrocytes were isolated from the fresh blood by centrifugation at 1500 rpm on a Janetzki centrifuge (Germany) in the presence of 3.8% sodium citrate and thrice washed with 0.9% solution of sodium chloride. In each experiment, a solution of peptide at the chosen concentration was prepared in a buffer (0.15 ml) containing 0.01 M Na_2HPO_4, 1.6 mM NaH_2PO_4, and 0.15 M NaCl (pH 5.0 or 7.8). Then, the erythrocyte suspension (10 μl) was added. The mixture was incubated for 1 h at 37°C at the occasional shaking. The hemolysis degree was determined according to the change in absorption of the supernatant at λ 492 nm after the removal of cells by centrifugation. We assumed the absorption values corresponding to 0% of hemolysis and to 100% hemolysis observed after the incubation of erythrocytes in the buffer solution and in

water, respectively. The given data are the average results of three independent experiments.

CD spectroscopy. CD spectra were recorded at room temperature. The measurements were made at the range of wavelength from 195 to 320 nm. The working peptide concentration was 30 μg/ml. Cuvettes with the lengths of optical path of 1 cm and 0.2 cm were used. The conformational states of peptides were estimated using the CDNN V2.1 program (Gerald Bohm, Institute für Biotechnologie, Wittenberg, Germany).

Preparation of the complexes of peptides with DNA of the pCMV*LacZ* plasmid and studies of their stability by the method of gel retardation. A solution (20 μl) containing various amounts of peptide was gradually added to an aqueous solution (20 μl) of plasmid DNA (1 μg). After 1-h incubation, the resulting complexes were analyzed by the method of gel retardation (according to decrease in the electrophoretic mobility of DNA in 0.8% agarose gel containing 2.7 μl of ethidium bromide in 400 g of dry agarose). The results presented in Fig. 5 are average from two independent experiments.

An analysis of stability of the plasmid DNA in the peptide complexes to the hydrolysis by nucleases. The efficiency of protection of the pCMV*LacZ* plasmid construct from the hydrolysis by DNase I was studied in the complexes with each synthesized peptide at different DNA–peptide charge ratio. DNase I (0.1 activity U) was added to the solutions of complexes and incubated for 1 h at 37°C. DNase was then inactivated by heating to 70°C for 15 min. The complex was treated with proteinase K and then subjected to the phenol–chloroform extraction [23]. The DNA state was evaluated by gel electrophoresis. The incompact DNA treated with DNase I and the native plasmid DNA were used as a negative (K–) and a positive (K+) controls, respectively. The average results of two independent experiments are given.

Evaluation of the efficiency of cell transformation by the peptide–DNA of the pCMV*LacZ* plasmid complexes (Fig. 6). The cells (15000 cells per well with 1 ml of the standard culture medium) were seeded into 24-well plates 24 h before the transfection of the complexes. The standard culture medium consisted of the RPMI medium, calf embryo serum FBS 10%, and gentamycin (0.01 μg/ml). The cells were washed with the RPMI medium, and the RPMI medium (500 μl) containing no serum was placed into each well 10 min before the addition of the DNA–peptide complexes. The cells were transformed by the addition of suspensions (200 μl) of the DNA–carrier complexes containing 5 μg of DNA. If the transformation was carried out in the presence of chloroquine, its concentration was 100 μM. The mixture was carefully mixed, and the plates with cells were incubated in a thermostat for 2 h at 37°C in the atmosphere of 5% CO_2. The cells were then washed with the RPMI medium and the standard culture medium (1 ml per well) was added. The plasmid DNA compacted with the EscortTM commercial carrier and the native DNA were used as a positive and a negative control, respectively.

The activity of β-galactosidase was measured 48 h after the introduction of complexes in the following way. The cells were fixed on the cultural plates by the treatment with 0.5% glutaraldehyde in PBS (1.7 mM KH_2PO_4, 5.2 mM Na_2HPO_4, and 150 mM NaCl, pH 7.5); washed with PBS containing 2 mM $MgCl_2$ and with the detergent solution (0.01% sodium deoxycholate, 0.02% Nonidet P-40, and 2 mM $MgCl_2$); and incubated for 16 h in a mixture of X-gal (2 mg/ml), $K_3Fe(CN)_6$ (5 mM), $K_4Fe(CN)_6$ (5 mM), and $MgCl_2$ (2 mM) in PBS. The plates were washed with PBS and inspected under the Zeiss phase-contrast microscope (Germany). The presence of β-galactosidase in the nuclei of transfected cells was determined according to the specific violet coloring. The portion of cells expressing the bacterial β-galactosidase was the criterion of the transfection efficiency of the peptide complexes. The data on the activity of β-galactosidase are the average result of three independent experiments.

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