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# Complexes of DNA with Cationic Peptides: Conditions of Formation and Factors Effecting Internalization by Mammalian Cells

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**Abstract**—This work was devoted to the study of conditions of the formation of DNA/K8 complex and analysis of factors effecting the entry of DNA/K8 complex into mammalian cells in comparison with DNA complexes with arginine-rich fragment (47-57) of human immunodeficiency virus (type 1) transcription factor Tat (Tat peptide). The stoichiometry of positively charged DNA/K8 complexes has been studied for the first time. Non-cooperative character of DNA–K8 interaction was revealed. It has been shown that along with the positive charge of such complexes, the presence of an excess of free K8 peptide in the culture medium is a necessary condition for maximal efficiency of cell transfection with DNA/K8 complexes. A stimulatory effect of free K8 peptide on the efficiency of mammalian cell transfection by DNA/K8 complexes is likely to be mediated by the interactions of cationic peptide K8 with negatively charged proteoglycans on the cell surface, which leads to protection of DNA/K8 complexes from disruption by cellular heparan sulfates. However, the protective role of free cationic peptides depends not only on their positive charge, but also on the primary structure of the peptide. In contrast with the results obtained for DNA complexes with molecular conjugates based on poly-L-lysine, the aggregation of DNA/K8 complexes leads to a significant increase in the expression of transferred gene.

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*Key words*: non-viral gene transfer approaches, cationic peptides, endocytosis, K8, Tat peptide

Elaboration of methods for DNA transfer into mammals is crucial for the development of approaches to genetic correction of human pathologies. Application of viral vectors for this purpose is quite limited [1, 2]. In this connection, the development of non-viral methods for DNA transfer is an important task. Despite the lower efficiency, non-viral means are free of certain drawbacks that are typical for viral vectors (high immunogenicity and toxicity) [3]. The most popular approach in this area is the use of cationic carriers for DNA condensation due to electrostatic interactions followed by the introduction of polyelectrolyte complexes DNA/carrier into mammalian cells *in vitro* or *in vivo* [4]. The most frequently used carriers are based on poly-L-lysine, polyethyleneimine, or cationic lipids representing the molecular conjugates of a cationic moiety and a ligand to cell receptors [3, 4]. The entry of DNA complexes with molecular conjugates into cells usually occurs through a receptor-mediated endocytosis, which involved receptors specific to the ligand used [4]. Despite the number of advantages of molecular conjugates (low toxicity, possibility of site-specific delivery to tissues and organs), their use is limited by relatively low efficiency of gene transfer. One of the main factors preventing high level of expression of transferred genes is the difficulty in release of DNA–conjugate complexes from endosome compartments. The major part of DNA remains in cellular endosomes and is degraded in the course of endosome maturation and fusion with lysosomes [5]. There are data available in the literature that describe the inverse dependence of the level of cell transfection by DNA/con-

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jugate complexes on the size of the complexes used [6]. These observations resulted, on one hand, in the decrease in size of ligand moiety comprising the molecular conjugates (for instance, the replacement of asialo-oromucoid with galactose residues) [6-8], and on the other hand in minimization of size of polycationic carriers, in particular, the replacement of poly-L-lysine with cationic oligopeptides containing 6-10 amino acid residues [9, 10]. Gottschalk and coauthors suggested using lysine-rich peptide K8 ($YKAK_8WK$), capable of compacting DNA with the formation of polyelectrolyte complexes, for DNA transfer into cells. The complexes can penetrate into mammalian cells and provide expression of transferred genes, despite the absence of any ligands to cell receptors in their composition [9, 11]. The mechanism of entry of DNA/K8 complexes remains unstudied; however, a number of data have been obtained indicating the involvement of endocytosis in this process [9]. We previously studied in detail the mechanisms of internalization of DNA complexes with arginine-rich fragment of human immunodeficiency virus (type-1) transcription factor Tat (Tat peptide) by mammalian cells [12]. The aim of this investigation was to study the conditions of formation of DNA complexes with K8, and elucidation of factors influencing the efficiency of the entry of such complexes into mammalian cells compared with DNA/Tat peptide complexes.

## MATERIALS AND METHODS

In this work we used human hepatocellular carcinoma (hepatoma) cell line HepG2 obtained from the American Type Culture Collection (ATCC, NIH), which is kept and maintained in a cell culture bank of the Institute of Cytology (Russian Academy of Sciences, St. Petersburg).

Plasmids pCMVL and pCMVluc carrying the gene of bacterial β-galactosidase (*lacZ*) and firefly luciferase *luc* under the control of promoter of human cytomegalovirus were described previously [11, 12].

Lysine-rich peptide $K_8$ ($YKAK_8WK$) [9], arginine-rich Tat peptide representing a fragment of the basic domain of transcription factor Tat ($^{47}$YGRKKRRQRRR$^{57}$) [13], and amphipathic peptide JTS-1 (GLFEALLELLESLWELLLEA) displaying lysing activity towards endosomes [9] were synthesized by the solid-phase method using the BOC/Bzl strategy on a NPS-4000 semi-automated synthesizer (Neosystem Laboratoires, France) on 4-methylbenzhydrylamino polymer (0.55 mmol/g; 0.25 mmol). Biotin or 5(6)-carboxyfluorescein was attached to peptides K8 and Tat through a glycine spacer [14]. Upon completion of the synthesis, the final product was detached from the polymeric support with simultaneous deblocking with a 1 M solution of trifluoromethanesulfonic acid in trifluoroacetic acid according to a standard protocol from Applied Biosystems (USA) [15]. The peptides were purified using gel filtration on Sephadex G-15 in 50% acetic acid followed by reversed phase high performance liquid chromatography (HPLC) on a Delta Pak $C_{18}$ column (Waters Chromatography, Division of Millipore, USA) in the system $H_2O$–acetonitrile–0.01% trifluoroacetic acid. In the case of JTS-1 a gradient of isopropanol in 0.01 M ammonium phosphate (pH 6.8) was applied.

The purity of the obtained products was monitored using analytical HPLC. Peptide structure was confirmed by amino acid analysis data (T 339 M analyzer, Mikrotechna, Czech Republic) and ESI-MS (95 XL MAT mass spectrometer, ThermoFinnigan, USA).

The formation of DNA complexes with K8, Tat, and their biotinylated analogs was performed in phosphate-buffered saline (PBS) using different DNA to peptide charge ratios in a reaction medium according to [11]. Generation of DNA/peptide complexes was monitored by the gel retardation method in agarose gel. In separate experiments after the completion of complex formation the biotinylated complexes were aggregated by the introduction of streptavidin into the reaction medium. JTS-1 peptide was incorporated into the complexes as described earlier [11]. To formed DNA/K8 complexes with DNA/peptide charge ratio of 1 : 2, JTS-1 peptide was added to the final concentration of 38 nM. The formed complexes were stirred and incubated for 30 min at room temperature. In the experiments on determination of sensitivity of DNA in the complex to DNase I (Fermentas, Lithuania), $MgCl_2$ up to a final concentration of 3 mM and 3 units of DNase I (on the basis of 1 unit of DNase I per 1 μg of DNA) were added to the reaction mixture (30 μl) after the completion of complex formation. The mixture was incubated at 37°C for 30 min. Complexes treated with DNase I were analyzed by gel retardation assay. The stoichiometry of DNA/K8 complexes were studied as described previously [12]. Complexes of DNA with K8 were prepared under peptide saturation conditions, followed by removal of unbound peptide by ultrafiltration using centrifugation on Centricon YM-10 membrane filters (Millipore). The amount of peptide in the complex was determined by a back titration with heparin.

HepG2 cells were transfected with DNA/K8, DNA/Tat, and DNA/K8-streptavidin preparations following the method suggested by Gottschalk et al. [9]. The cells were plated on a Petri dish (diameter 30 mm) with the density of $10^4$ cells/cm$^2$ and grown up to 60-70% of monolayer in DMEM medium containing 10% fetal bovine serum (FBS) (Biolot, Russia) at 37°C in a humidified atmosphere containing 5% $CO_2$. DNA/peptide complexes with different DNA to peptide charge ratios were prepared on the basis of 12 μg of DNA in 500 μl of PBS per Petri dish. After incubation of cells with complexes for 2 h, non-internalized complexes were washed from the cells with heparin solution (58 μg/ml in PBS) and incubated in DMEM medium containing 10% FBS during 24 h.

Activity of bacterial β-galactosidase in cells was determined by a standard method using chromogenic substrate *o*-nitrophenyl-β-D-galactopyranoside (ONPG) supplied by Sigma (USA) as described in [16]. Activity of luciferase was measured using a commercial luciferase assay from Promega (USA) (Luciferase Assay System, cat. number E4030) following the manufacturer's instructions. Luminescence was measured on a β-scintillation counter (Beckman Instruments, USA). Protein concentration in cell lysates was determined by the Bradford method [17]. β-Galactosidase activity was calculated as the ratio of optical density $A_{420}$ per mg of total protein during 1 h of reaction. Luciferase activity was expressed in relative light units (RLU) representing number of emissions per min per mg of total protein in the cell extract.

Statistical data treatment was performed using the Statistica Release 5.0 software package provided by StatSoft, Inc. The diagrams show average values of 3-5 replicates. Error bars correspond to standard error of mean.

## RESULTS AND DISCUSSION

**Character of formation of DNA complexes with K8.** Earlier we demonstrated the efficiency of delivery of plasmid DNA into mammalian cells using synthetic cationic lysine-rich peptide K8 and arginine-rich fragment of the human immunodeficiency virus type-1 Tat (Tat peptide) [11, 12]. Preliminary data indicated similarity in the entry pathways for DNA/K8 and DNA/Tat into mammalian cells. The DNA/peptide ratio during complex formation had a significant effect on the efficiency of transfection. Moreover, in the case of Tat peptide the stimulating effect of excess free peptide on the entry of DNA/Tat complexes into mammalian cells was demonstrated. In regard to K8, such information was not available in the literature. It appeared interesting to study the conditions for formation of complex of plasmid DNA and K8, and to elucidate the factors influencing the efficiency of DNA transfer in the complex with K8 into mammalian cells. DNA complexes with K8 and also well-studied polyelectrolyte complexes of DNA with poly-L-lysine are formed as a result of electrostatic interaction between polycation and DNA, which results in a decrease in negative charge of DNA, and, therefore, to the decrease in mobility of DNA on gel electrophoresis. According to the literature, complete retardation of DNA in the start wells as DNA/polycation complexes corresponds to neutralization of DNA charge and occurs upon the molar ratio of nucleotide/ε-amino group of lysine of 1 : 1 [6, 7, 18, 19]. Apparently, upon the further increase in polycation content in the complex the charge becomes positive. The formation of DNA/K8 complexes was performed under isotonic conditions (150 mM NaCl) at room temperature. Unlike poly-L-lysine, where high ionic strength of the reaction medium (1.1 M NaCl) is necessary to avoid aggregation of complexes with DNA [8, 20], binding of DNA to K8 under isotonic conditions is not accompanied by the formation of insoluble particles. Figure 1 shows the results of analysis of DNA/K8 complexes obtained using the increasing ratio of positive charge of the peptide to negative charge of DNA using the gel retardation method. In our experiments on titration of plasmid DNA (pCMVL) with increasing amounts of K8 the complete neutralization of negative charge of DNA, estimated by mobility in the electric field, is observed at the molar ratio of DNA to K8 equal to 1 : 1716, which is similar to the theoretical value (lane *5*). This method does not allow estimating maximal possible content of K8 in positively charged complexes prepared with molar excess of K8 relative to DNA. The stoichiometry of DNA/K8 complexes was investigated by purification of the complexes formed in the presence of a saturating amount of K8 (ten-fold excess by charge) from unbound peptide using the ultrafiltration method. As a result, complex containing a saturating amount of K8 was isolated. It is known that heparin, being a stronger polyanion than DNA, is capable of displacing DNA from its complexes with polycations [21, 22]. Determination of DNA content in the isolated complex was carried out spectrophotometrically after its complete destruction with an excess of heparin. The content of peptide in the studied complex was determined by a back titration with heparin. For this purpose, a calibration curve for the dependence of heparin amount, sufficient for the complete disruption of DNA/K8 complex, on the amount of K8 in the reaction medium during the formation of complexes was plotted (Fig. 2a). It was established that the maximal possible amount of K8 in the complex corresponds to a 1.8-fold excess of positive charge of K8 relative to negative charge of DNA. We earlier obtained similar results for the DNA complex with Tat peptide [12].

Interaction of DNA with polycations results in its compaction, and, as a result, in protection from nucleases [20, 23]. Moreover, the degree of DNA compaction in the complex directly correlates with its sensitivity to nucleases. Figure 3 presents the results of treatment of DNA/K8 complexes, prepared using different DNA to peptide

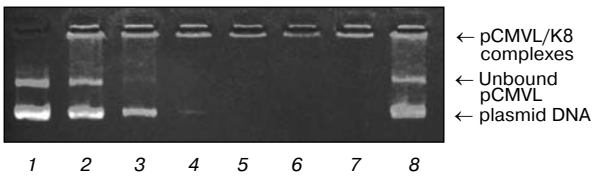


**Fig. 1.** Formation of complexes K8/pCMVLacZ. Optimization of molar ratio using the gel retardation method. Lanes: *1*) DNA/K8 charge ratio 1 : 0.15; *2*) DNA/K8 ratio 1 : 0.25; *3*) DNA/K8 ratio 1 : 0.7; *4*) DNA/K8 ratio 1 : 0.9; *5*) DNA/K8 ratio 1 : 1; *6*) DNA/K8 ratio 1 : 1.5; *7*) DNA/K8 ratio 1 : 2; *8*) naked pCMVLacZ DNA.

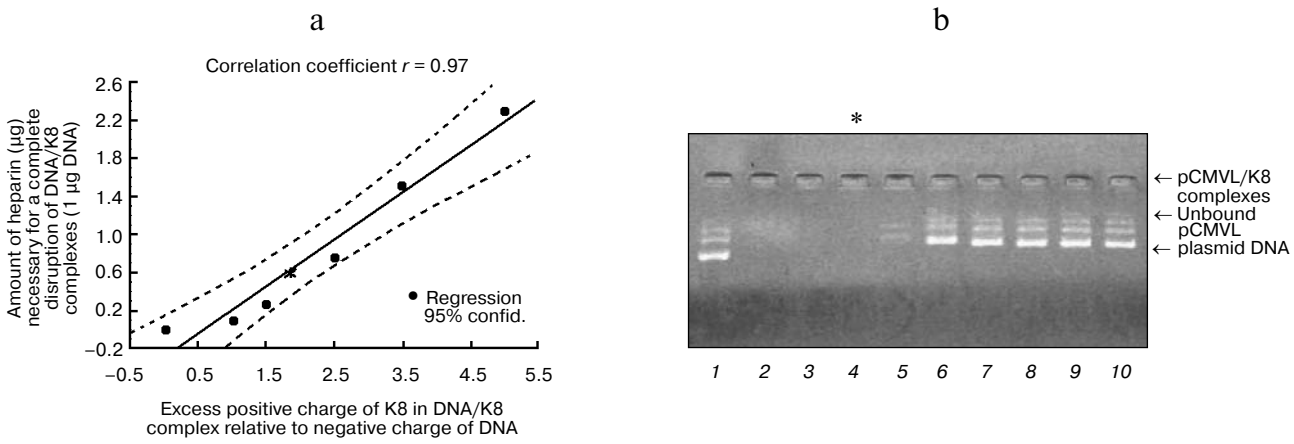


**Fig. 2.** Determination of maximal possible content of K8 in pCMVL/K8 complexes. a) Calibration curve for the dependence of heparin amount required for complete disruption of pCMVL/K8 complexes on the charge ratio of DNA to K8 in the reaction medium during the complex formation. b) Titration with heparin of K8 in pCMVL/K8 complex (1 μg pCMVL) obtained using saturating amount of K8 and purified from unbound peptide by ultrafiltration: *1*) pCMVL; *2*) without heparin; *3*) with 0.292 μg heparin; *4*) with 0.4 μg heparin; *5*) with 0.52 μg heparin; *6*) with 0.65 μg heparin; *7*) with 0.975 μg heparin; *8*) with 1.46 μg heparin; *9*) with 2.19 μg heparin; *10*) with 3.29 μg heparin. Asterisks show position on the calibration curve that corresponds to a complete destruction of the complex by heparin (a) and an experimental data point corresponding to a complete destruction of the complex by heparin revealed by gel retardation assay (b).

charge ratio, with DNase I. As expected, increase in peptide content in the reaction medium leads to a better protection of DNA from degradation. DNA in complexes prepared with the ratio of negative DNA charge and positive peptide charge of 1 : 1 (electrically neutral complexes) is only partially protected from degradation (Fig. 3, lane *4*). Total resistance to the action of DNase I is observed only in the complexes obtained with charge ratio of less than 1 : 1.5. The data are in a good agreement with the results of stoichiometry study of DNA/K8 complexes (Fig. 2), where the maximal K8 content in the complex with DNA corresponded to a charge ratio of 1 : 1.8.

Estimation of sensitivity of DNA/K8 complexes to DNase I revealed another important parameter of complex formation, the cooperativity of DNA–polycation interaction. Two models of complex formation are possible. In the case of cooperative interaction negatively charged DNA/polycation complexes display higher affinity to free polycation than unbound DNA. Therefore, if the DNA/polycation charge ratio is higher than 1 : 1 (excess of DNA), the reaction medium will contain a mixture of electrically neutral (or bearing small positive charge) complexes and free DNA molecules. In the case of non-cooperative interaction, polycation molecules are statistically (randomly) distributed among DNA molecules, and as a result, the reaction medium will contain a more or less homogeneous mixture of complexes, whereas their charge will be determined by the initial ratio of DNA to polycation [20]. With the cooperative character of DNA interaction with polycation, the treatment of complexes obtained at the charge ratio higher than 1 : 1 with DNase I will lead to a complete disruption of unbound DNA in the reaction medium (on one hand), and to a total protection of DNA in the complex with polycation (on the other hand). When it comes to non-cooperative interaction, treatment of negatively charged complexes with DNase I will result in a partial degradation of all DNA, whereas the size of the degradation products will increase with the increase in polycation content in the complex. The dependence of resistance of DNA in the complex with K8 to DNase I on DNA to peptide charge ratio corresponds to non-cooperative interaction (Fig. 3).

**Effect of free cationic peptides K8 and Tat on internalization of DNA/K8 complexes by mammalian cells.** Previously it was shown that DNA/polycation complexes obtained under the conditions of an excess of positive charge have significantly higher transfection efficiency. Usually, higher level of cell transfection in the case of such complexes is explained by a better adsorption of pos-

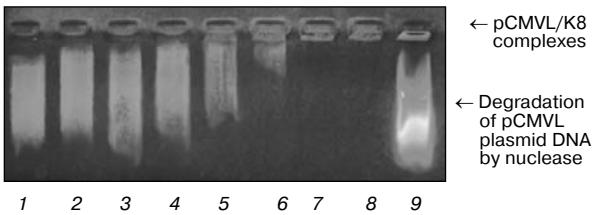


**Fig. 3.** Effect of DNase I on DNA/K8 complexes obtained with different pCMVL/K8 ratios: *1*) DNA/K8 charge ratio 1 : 0.25; *2*) charge ratio 1 : 0.5; *3*) charge ratio 1 : 0.75; *4*) charge ratio 1 : 1; *5*) charge ratio 1 : 1.25; *6*) charge ratio 1 : 1.5; *7*) charge ratio 1 : 1.75; *8*) charge ratio 1 : 2; *9*) free pCMVL. Arrows show pCMVL/K8 complexes and degradation products of the plasmid DNA by DNase I.

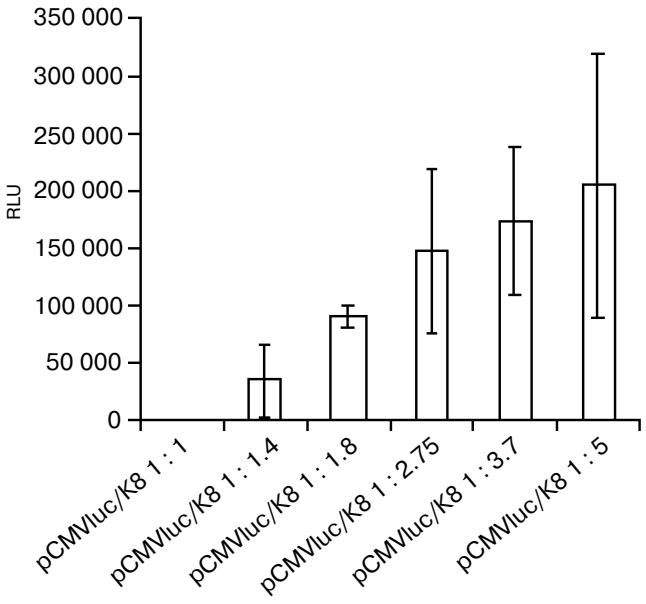


**Fig. 4.** Estimation of transfection efficiency of HepG2 cells with pCMVluc/K8 complexes using different ratios of DNA/K8 charge ratio. The level of luciferase activity is expressed in relative light units (RLU). Charge ratios are given below the corresponding bars.

itively charged DNA/polycation complexes on a negatively charged cell membrane [8, 24, 25]. Nevertheless, earlier we succeeded in showing a stimulating effect of free Tat peptide on the entry of DNA/Tat complexes into cultured mammalian cells [12]. Figure 4 presents the results of analysis of transfecting activity of DNA/K8 complexes obtained using different charge ratios of DNA to peptide. Acquisition of a positive charge by the complex leads to a dramatic increase in transfection efficiency. With DNA/peptide charge ratio of 1 : 1.8 complexes are formed with the maximal possible positive charge; further increase in peptide content in the reaction medium during the complex formation leads to the presence of excess of free K8 not bound to DNA (see above). Nevertheless, transfection efficiency of such complexes gradually increases as far as DNA to K8 charge ratio decreases. Hence, as in the case of Tat peptide, free K8 is capable of stimulating cell transfection with DNA/K8 complexes. An excess of unbound K8 can have a protecting effect through its binding to heparin sulfates on a cell surface, thus protecting DNA/peptide complexes from disruption, similarly to an excess of positively charged Tat peptide [12]. Two different mechanism of such effect are possible. In the first case, the process has low specificity and its efficiency is determined entirely by cationic properties of the peptide: the higher the density of the positive charge, the stronger the protective effect will be. The second alternative implies, along with purely electrostatic forces, the existence of a certain stereospecificity between proteoglycan chain and cationic peptide. In this case the stimulating effect of free peptide on the efficiency of cell transfection with DNA/peptide complexes will be dependent not only on the density of peptide positive charge, but also on its structure. Cationic peptides K8 and

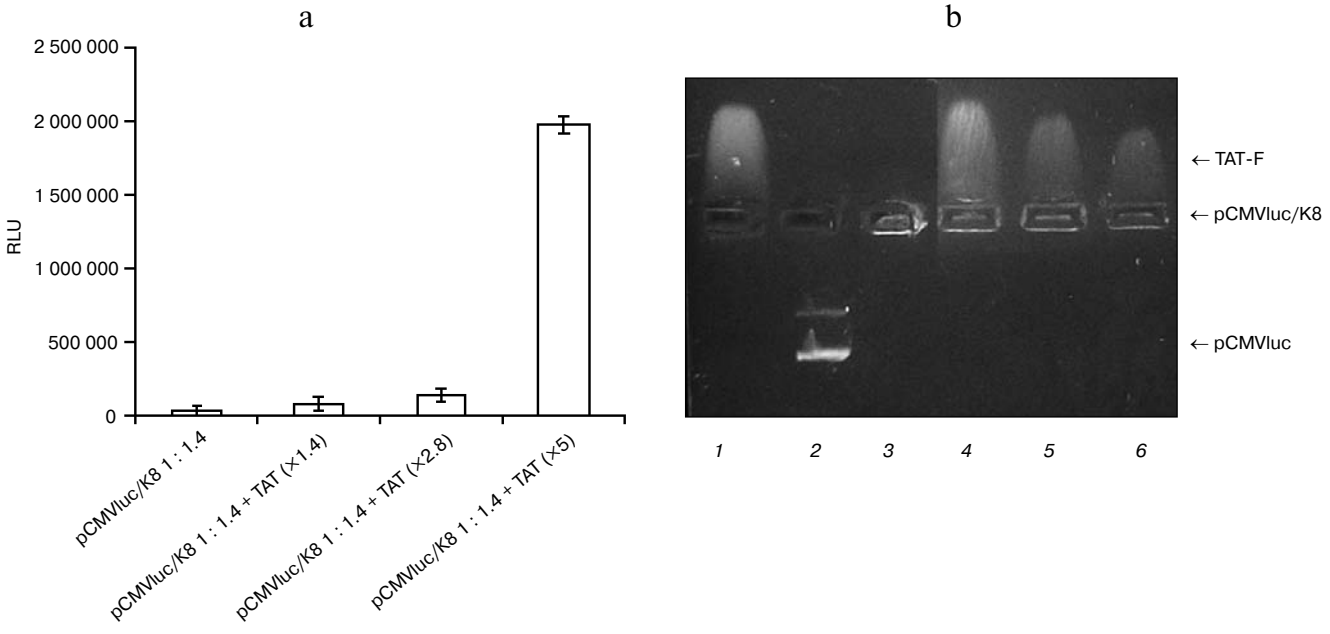


**Fig. 5.** Effect of Tat peptide on pCMVluc/K8 complexes: a) influence of Tat peptide on the efficiency of HepG2 cell transfection with pCMVluc/K8; b) effect of Tat peptide on the stability of pCMVluc/K8. To pCMVluc/K8 complexes (DNA/peptide charge ratio 1 : 1.4) the specified excess (by charge) of fluorescent labeled Tat peptide (Tat-F) was added, incubated for 10 min, and analyzed using gel electrophoresis: *1*) free Tat-F; *2*) free pCMVluc; *3*) pCMVluc/K8; *4*) pCMVluc/K8 + Tat-F (×5); *5*) pCMVluc/K8 + Tat-F (×2.8); *6*) pCMVluc/K8 + Tat-F (×1.4).

Tat have practically the same density of positive charge (molar weight of a single positive charge is 245 and 240 g, respectively). Moreover, their ability to bind to DNA is almost identical. Therefore, upon nonspecific interaction of peptide with proteoglycan the stimulating effect of the excess K8 and Tat peptide on the extent of cell transfection with DNA/peptide complexes should be approximately the same. To test this assumption, we performed a comparative study of the effect of excess of K8 and Tat peptide on the efficiency of transfection of human hepatoma HepG2 cells with DNA/K8 complexes. The results are presented in Fig. 5a. The excess of free Tat peptide was approximately 8 times more effective compared to the respective excess of K8. The possibility of displacement of K8 peptide from the complex with DNA under the conditions of the excess of free Tat peptide was verified experimentally using gel retardation of DNA/K8 complexes after their incubation with fluorescent labeled Tat peptide (Fig. 5b). The fluorescent label migrated to the cathode and was not retained in the wells together with DNA. Therefore, Tat peptide did not displace K8 from complexes with DNA. In this way, the results indicate that the Tat peptide has significantly higher affinity to negatively charged proteoglycans of the cell surface compared to K8. The data are in good agreement with previously published results of others on the high affinity of Tat peptide to heparin sulfates [27].

**Endocytosis-mediated internalization of DNA/K8 complexes by mammalian cells.** The mechanism of internalization of DNA/K8 complexes by cells remains virtually unstudied. Earlier we demonstrated the endocytosis-mediated uptake of DNA complexes with Tat peptide by mammalian cells. The system for plasmid DNA transfer suggested for the first time by Gottschalk et al. [9] included a synthetic amphipathic peptide JTS-1 facilitating the release of complexes from endosomes. This also implies the entry of complexes into cells by an endocytosis mechanism [9]. The stimulating effect of JTS-1 on the level of expression of a transferred receptor gene, therefore, confirms endocytosis-mediated internalization of DNA/K8 complexes by cells (Fig. 6a). Further evidence of the role of endocytosis in the entry of DNA/K8 complexes into HepG2 cells was obtained using chloroquine, an agent that inhibits maturation of early endosomes. Cell treatment with chloroquine leads to the stimulation of expression of a transferred reporter gene (Fig. 6b).

**Effect of the size of DNA/K8 complexes on the efficiency of their internalization by mammalian cells.** To elucidate the effect of the size of DNA/K8 complexes on the efficiency of HepG2 cell transfection with DNA/K8 complexes we used biotinylated peptide K8. Such modification does not influence the DNA-binding properties of K8 and the extent of cell transfection (data not shown). Addition of streptavidin to the complexes results in their aggregation (each streptavidin molecule contains four biotin binding sites). The aggregates appeared to be significantly more active compared to conventional DNA/K8 complexes (Fig. 7). The results are rather unexpected, since the common practice for the development of non-viral systems of DNA delivery based on cationic carriers is the intention to decrease the size of DNA/polycation complexes [4]. The explanation of this discrepancy is obviously concluded in the absence of specific receptors for DNA/K8 complexes on a cell surface. This implies the involvement of adsorption/endocytosis in the uptake of DNA/K8 complexes, contrary to DNA complexes with molecular conjugates that enter into cells through a receptor-mediated pathway [3, 4]. Another explanation of the higher transfection efficiency of the aggregates might be steric protection of DNA/K8 complexes by streptavidin, which prevents the contacts with negatively charged proteoglycans on the cell surface. Indirect evidence of this hypothesis is the results of experiments on the addition of Tat peptide to the complexes (Fig. 7). The addition of Tat peptide has no effect on the extent of cell transfection with such complexes, contrary

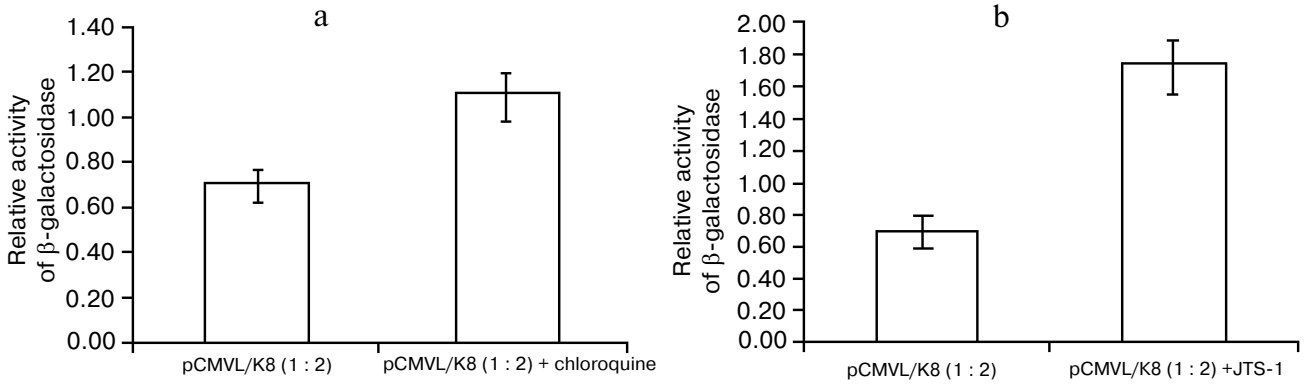


**Fig. 6.** Endocytosis-mediated internalization of DNA/K8 complexes in HepG2 cells: a) effect of chloroquine on the level of expression of *lacZ* gene marker; b) effect of amphipathic peptide JTS-1 with pH-dependent endolytic activity on the level of expression of *lacZ* gene marker.

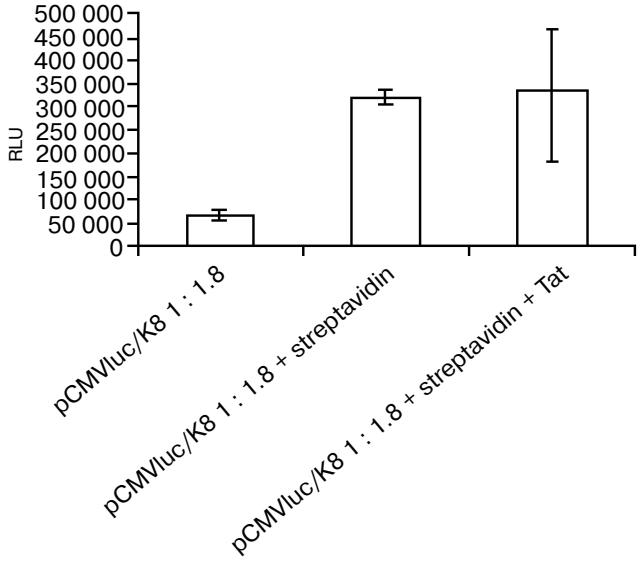


**Fig. 7.** Effect of aggregation of DNA/K8 complex by streptavidin on the efficiency of transfection of HepG2 cells. pCMVluc/K8 1 : 1.8, cell transfection with pCMVluc/K8 complexes; pCMVluc/K8 1 : 1.8 + streptavidin, cell transfection with pCMVluc/K8 complexes aggregated by streptavidin; pCMVluc/K8 1 : 1.8 + streptavidin + Tat, cell transfection with pCMVluc/K8 complexes aggregated by streptavidin in the presence of Tat peptide. The level of luciferase activity is expressed in relative light units (RLU).

to the previously described results on the stimulation of transfecting activity of DNA/K8 complexes by Tat peptide (Fig. 5). Apparently, DNA/K8/streptavidin conjugates do not need to be further protected from proteoglycans, which is expressed by the absence of Tat peptide effect on their transfection efficiency.

In the present work, the factors influencing the efficiency of delivery of plasmid DNA in a complex with cationic peptide K8 into mammalian cells were investigated. For the first time the stoichiometry of positively charged DNA/K8 complexes has been studied. Noncooperative interaction of DNA–K8 during the complex formation has been revealed. It is shown that along with a positive charge of such complexes, the presence of excess amount of free (not bound to DNA) peptide that provides protection of DNA/K8 complexes from destruction with negatively charged proteoglycans on a cell surface is necessary for displaying maximal transfection efficiency. It is determined that besides the positive charge the protecting effect is specified by the structure of the peptide. In particular, the addition of Tat peptide leads to a significantly increased extent of cell transfection compared to K8. Contrary to DNA complexes with molecular conjugates based on poly-L-lysine, aggregation of DNA/K8 complexes increases the level of expression of a transferred gene.

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