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Influenza virus hemagglutinin HA-2 N-terminal fusogenic peptides augment gene transfer by transferrin–polylysine–DNA complexes: Toward a synthetic virus-like gene-transfer vehicle

(gene therapy/DNA transfection/endocytosis/endosome disruption/transferrin receptor)

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ABSTRACT **Complexes containing plasmid DNA, transferrin–polylysine conjugates, and polylysine-conjugated peptides derived from the N-terminal sequence of the influenza virus hemagglutinin subunit HA-2 have been used for the transfer of luciferase or β-galactosidase marker genes to K562 cells, HeLa cells, and BNL CL.2 hepatocytes. These DNA complexes mimic the entry of viruses into cells, as they contain functions for (*i*) the packaging of the nucleic acid with polylysine, (*ii*) the attachment to the cell and receptor-mediated endocytosis with transferrin as a ligand, and (*iii*) the release from endosomes by using membrane-disrupting influenza peptides. The presence of these influenza peptide conjugates in the DNA complexes renders the complexes active in membrane disruption in a liposome leakage assay and results in a substantial augmentation of the transferrin–polylysine-mediated gene transfer.**

The high gene-transfer efficiency of viruses has led to the development of viral vectors in which part of the viral genome has been replaced by a gene to be introduced into eukaryotic cells. These recombinant viral vectors such as adenoviral (1, 2), retroviral (3, 4), vaccinia (5, 6), and parvoviral (7, 8) vectors have displayed useful gene delivery properties in a large number of applications. However, these vectors are limited in their cell tropism, in the size of the gene to be introduced, and in that the introduced DNA must be compatible with viral replication. Our approach to establish an efficient gene transfer device is based on the concept of adopting distinct viral entry functions and combining them in a modular fashion.

Viruses contain core proteins in their interior that package their nucleic acid genome into small compact structures. The surface proteins of many viruses (for instance adenoviruses, alphaviruses, orthomyxoviruses, or picornaviruses) contain two further functions essential for the delivery of the viral genome into the cytoplasm of the target cells: (*i*) the binding of the virus particle to a cellular surface receptor that mediates endocytosis of the virion into endosomes, and (*ii*) after endocytosis, the release of the viral genome from endosomes into the cytoplasm by disruption of the endosomal membrane, in the case of membrane-free viruses (such as adenoviruses), or the fusion of the viral membrane with endosomal membranes, in the case of enveloped viruses (such as influenza virus). Viruses that enter via this endosomal route are thought to exploit the endosomal acidification process, which specifically activates their endosomolytic or fusogenic protein domains. This has been demonstrated in detail for the influenza viruses (9).

We, and others (10, 11), have used the cellular mechanism of receptor-mediated endocytosis of macromolecules or viruses, employing ligands such as transferrin (12–14) or viral proteins (15), for the import of DNA molecules into cells. The key steps have been the conjugation of the ligand for a certain cell receptor to a DNA-binding moiety, such as a polycation or an intercalating compound, and the formation of complexes of this conjugate with the DNA to be delivered. We have demonstrated that polylysine conjugates condense the DNA into small structures with a size of 80–100 nm (14), which approximate the size of many viruses. These DNA complexes, however, still lack the second important function of viral entry—namely, a mechanism for escape from endosomes, which is a limiting step for reporter gene expression during receptor-mediated gene delivery (16).

In the present communication, we describe the inclusion of fusogenic peptides derived from the N-terminal sequence of the influenza virus hemagglutinin subunit HA-2 as part of the DNA complexes and the resulting augmentation of gene transfer to cultured cells. These DNA complexes, containing polylysine as the nucleic acid packaging module, polylysine-modified transferrin as the receptor binding module, and polylysine-bound influenza peptides as the endosomolytic module, represent a simple model of an artificial membrane-free virus without replication functions, which can be used to deliver genes to eukaryotic cells.

MATERIALS AND METHODS

Materials. Human transferrin–poly(L-lysine) conjugates with an average chain length of 190 lysine residues (TfpL190B) or 290 lysine residues (TfpL290) were prepared as described (17). N^{ε}-undecyl-polylysine (C11pL) was synthesized by reaction of the trifluoroacetate salt of poly(L-lysine) (average chain length of 200 lysine monomers, prepared from hydrobromide salt; Sigma) in a solution of freshly distilled dioxane/water, 4:1 (vol/vol), with undecanal (Sigma) and reduction of the resulting aldiminium salts to the corresponding amines with sodium cyanoborohydride. According to NMR analysis, polylysine was modified with about 50 undecyl groups per molecule.

Peptide Synthesis. Peptide 1 with the sequence Gly-Leu-Phe-Glu-Ala-Ile-Ala-Gly-Phe-Ile-Glu-Asn-Gly-Trp-Glu-Gly-Met-Ile-Asp-Gly-Gly-Gly-Cys was synthesized by the fluoren-9-ylmethoxycarbonyl (Fmoc) procedure (18) using an Applied Biosystems 431A peptide synthesizer. The crude peptide dissolved in 3.5 ml of 20 mM aqueous ammonium bicarbonate containing 300 μl of 25% ammonia per liter (buffer A) was purified by ion-exchange chromatography (Mono Q column;

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Abbreviations: TfpL190B and TfpL290, transferrin–poly(L-lysine) conjugates with an average chain length of 190 and 290 lysine residues, respectively; C11pL, N^{ε}-undecyl-polylysine; $pLys_{300}$, polylysine with an average chain length of 300 lysine residues; Influ1pL, Influ2pL, and Influ3pL, conjugates of polylysine with influenza peptide 1, peptide 2, and peptide 3, respectively; FCS, fetal calf serum.

Pharmacia; gradient elution with 0–100% buffer B in buffer A; buffer B = buffer A plus 3 M NaCl; the peptide was eluted at 1 M NaCl) and subsequently by reverse-phase HPLC (Bio-Rad Hi-Pore RP-304 column; gradient elution with 50–100% buffer C in buffer A; buffer C = buffer A in 98% methanol) to yield pure peptide 1 in the cysteine-protected form. A time-of-flight mass spectrum (19) obtained with a Bio Ion ^{252}Cf PD-MS instrument (Uppsala, Sweden) showed a mass of 2401 ($M + H^+$). Peptide 1 in the free mercapto form was obtained by treatment with thioanisol/ethanedithiol/trifluoroacetic acid/trifluoromethanesulfonic acid, 2:1:40:3 (vol/vol).

Peptide 2 (with Glu-4 of peptide 1 changed to Gly) was synthesized in a manner analogous to peptide 1. A control peptide 3 with the same amino acid composition as peptide 1, but with the scrambled sequence Phe-Leu-Gly-Ile-Ala-Glu-Ala-Ile-Asp-Ile-Gly-Asn-Gly-Trp-Glu-Gly-Met-Glu-Phe-Gly-Gly-Gly-Cys, was also synthesized.

Conjugation of Influenza Peptides to Polylysine. Polylysine with an average chain length of 300 lysine residues ($pLys_{300}$; 67 nmol, modified with 0.3 μmol of 2-pyridyldithiopropionate linker, prepared as described in ref. 12) in 2.2 ml of 2 M guanidinium hydrochloride/20 mM ammonium bicarbonate, pH 8.2, was allowed to react overnight at room temperature with 0.35 μmol of peptide 1 in the thiol form. The mixture was dialyzed against 0.5 M guanidinium hydrochloride/20 mM Hepes, pH 7.3. Conjugates were isolated by cation-exchange chromatography on a Mono S column (Pharmacia) using a gradient from 0% to 100% buffer E in buffer D (buffer D = 0.5 M guanidinium hydrochloride, 20 mM Hepes, pH 7.3; buffer E = 3 M guanidinium hydrochloride/20 mM Hepes, pH 7.3). The material eluting with 1.5 M guanidinium hydrochloride was pooled and dialyzed against HBS (150 mM NaCl/20 mM Hepes, pH 7.3). According to a ninhydrin assay and absorption at 280 nm, influenza peptide 1–polylysine conjugates (Influ1pL) with a molar ratio of peptide to $pLys_{300}$ of 4:1 were obtained. The coupling of the influenza peptide 2 or peptide 3 to polylysine was performed as described above. Influenza peptide 2–polylysine conjugates (Influ2pL) with a molar ratio of peptide to $pLys_{300}$ of 1:1, 3:1, or 8:1 were synthesized.

Liposome Leakage Assays. Liposomes of phosphatidylcholine (Avanti Polar Lipids) containing 100 mM calcein (adjusted to pH 7.3 by addition of sodium hydroxide) were prepared by using the reverse-phase evaporation method (20, 21). The liposome mixture was extruded several times through a Nucleopore polycarbonate membrane (100 nm). The liposomes were separated from nonincorporated material by gel filtration (Sephadex G-50; Pharmacia). The lipid phosphorus of the resulting liposome solution was determined by using the method of Bartlett (22). Leakage of liposomal contents in the presence of various lysogenic peptides or derivatives was measured by the release of entrapped calcein (23). The calcein fluorescence was measured at 515 nm (excitation at 490 nm). Aliquots of the above liposome solution were diluted 100-fold (final concentration, 25 μM lipid) with 0.15 M sodium chloride/citrate buffers of the appropriate pH (4.5–7.3). To 1 ml of these solutions was added 0.5 μg of peptide (*t*-butyl-protected form at 0.2 μg/μl in HBS), while mixing with a gentle stream of argon (final concentration, 0.2 μM peptide). The values corresponding to 100% leakage were determined by complete lysis of liposomes after the addition of 5 μl of 10% Triton X-100 (Fluka).

Cells, DNA Complex Formation, and Transfections. K562 cells were grown in suspension in RPMI 1640 medium (GIBCO/BRL) containing 10% fetal calf serum (FCS), penicillin at 100 units/ml, streptomycin at 100 μg/ml, and 2 mM glutamine. At 20 hr before transfection, the cells were placed in fresh medium containing 50 μM desferrioxamine (13). Transfections were performed with 5×10^5 cells suspended in 2 ml of fresh medium containing 10% FCS (plus 50 μM desferrioxamine). HeLa cells were grown in 6-cm plates in Dulbecco's modified Eagle's medium plus 5% FCS, antibiotics, and glutamine. Transfections were performed at a density of 3×10^5 cells per plate with 1 ml of fresh medium containing 2% FCS. For β-galactosidase gene transfer experiments, HeLa cells were grown in 3-cm culture dishes on coverslips (3×10^4 cells per dish). BNL CL.2 cells were grown in 6-cm plates in high-glucose Dulbecco's modified Eagle's medium (0.4% glucose) supplemented with 10% FCS, antibiotics, and glutamine. Approximately 1 hr before the transfection, the cells were cultured in 1 ml of fresh medium containing 2% FCS.

DNA complexes were prepared by mixing a solution of 6 μg of plasmid DNA in 330 μl of HBS with a solution of 12 μg of TfpL190B (or 8 μg of TfpL290) in 160 μl of HBS. DNA combination complexes containing transferrin–polylysine and influenza peptide–polylysine conjugates were prepared by first mixing 6 μg of plasmid DNA in 160 μl of HBS with 6 μg of TfpL190B (or 4 μg of TfpL290) in 160 μl of HBS and, after 15–30 min at room temperature, subsequent mixing with the influenza peptide–polylysine conjugate in 160 μl of HBS. Addition of the DNA complexes to the cells and further treatment of the cultures is described in the figure legends. Cells were harvested and assayed for luciferase activity as described (12, 15). β-Galactosidase activity was assayed as described in refs. 24 and 25.

RESULTS

Synthesis and Properties of Membrane-Disruptive Peptide–Polylysine Conjugates. We synthesized two peptides containing the sequences of the 20 N-terminal residues of HA-2: peptide 1 with a substitution of glycine for glutamic acid at position 4 from a mutant sequence (26) and peptide 2 with a wild-type sequence, similar to that described in ref. 27, but with C-terminal extensions containing a terminal cysteine as a handle for conjugation. The membrane-disrupting properties of these peptides were determined by a liposome leakage assay, which measures the release of calcein, a fluorescent dye, from phosphatidylcholine liposomes (Fig. 1*A*). Peptide 1 showed leakage activity only at acidic pH,

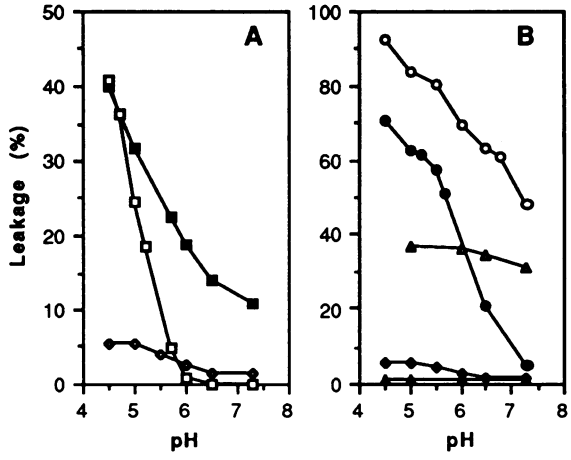

FIG. 1. Liposome leakage mediated by influenza peptides, peptide–polylysine conjugates, or peptide–polylysine conjugates complexed to DNA. Leakage activity was determined by mixing the compound with a solution of calcein-loaded liposomes and measuring the fluorescence after a 20-min incubation at room temperature (see *Materials and Methods*). (*A*) □, 0.5 μg of peptide 1; ■, 0.5 μg of peptide 2; ◇, 0.5 μg of peptide 3. (*B*) ○, Influ1pL (0.5 μg of peptide 1 linked to 3.3 μg of polylysine); ◆, Influ3pL (0.5 μg of peptide 3 linked to 3 μg of polylysine); ●, Influ1pL conjugates (0.5 μg) complexed with plasmid DNA (1 μg) and TfpL290 (0.7 μg); △, C11pL (3.3 μg); ▲, unmodified polylysine (3.3 μg).

whereas peptide 2, derived from the wild-type sequence, had some residual leakage activity at neutral pH. These leakage data are consistent with liposome fusion data of analogous peptides (27). We have observed an increased tendency of the wild-type sequence (peptide 2) to lose some of the leakage activity upon storage at 4°C in physiological buffer at pH 7.3, which may be explained by partial self-aggregation. In contrast, the acidic mutant peptide 1 does not display this behavior.

The peptides were linked to polylysine by reacting the cysteine mercapto groups with pyridyldithio-modified polylysines to yield disulfide-conjugated peptide–polylysine derivatives. The conjugates Influ1pL, derived from peptide 1, and Influ2pL, derived from peptide 2, with one to eight influenza peptides per polylysine chain were prepared (see *Materials and Methods*). In the liposome leakage assay, the peptide conjugate Influ1pL had a higher activity than unconjugated peptide 1 (Fig. 1*B*). The conjugate Influ1pL still showed a pH dependence but, in contrast to free peptide 1, had significant activity at neutral pH. Unmodified polylysine had no leakage activity; polylysine conjugated to the control peptide 3 (Influ3pL) had very low activity. A lipophilic polylysine, modified with undecyl groups (C11pL), showed a moderate, pH-independent activity in this assay (Fig. 1*B*). Complexing the peptide–polylysine conjugate with DNA reduced the leakage activity again, in particular the activity at neutral pH (Fig. 1*B*).

Luciferase Gene Delivery by DNA Combination Complexes Containing Transferrin and Membrane-Disruptive Peptides. Combination complexes consisting of plasmid DNA encoding the *Photinus pyralis* luciferase (pCMVL) as a reporter gene, transferrin–polylysine, and peptide–polylysine conjugates were prepared by adding transferrin–polylysine to DNA to neutralize half of the DNA charge; the remainder of the charge was then saturated by addition of influenza peptide–polylysine conjugate. The standard transferrinfection to the erythroleukemic cell line K562 requires the presence of chloroquine (13). In the absence of this lysosomatropic agent, no subsequent gene expression occurred with either transferrin–polylysine-complexed DNA or polylysine-complexed DNA (Fig. 2). The combination complexes containing the influenza peptide conjugate, however, particularly if transferrin–polylysine was included as the receptor-binding component, showed high luciferase expression (Fig. 2). Neither unconjugated peptide (data not shown) nor polylysine modified with lipophilic undecyl residues (Fig. 2) can substitute for the polylysine-conjugated peptide in mediating gene expression. This indicates that the amphipathic character of the influenza peptide conjugates cannot be replaced by lipophilic modifications, although these modifications allow polylysine to disrupt membranes to a moderate degree, albeit in a pH-independent manner (see Fig. 1).

Similar gene transfer experiments were performed with HeLa cells that, even in the presence of chloroquine, showed only a moderate transferrinfection efficiency. The data shown in Fig. 3 demonstrate the importance of including the peptide conjugate in the DNA–transferrin–polylysine complex. Incorporation of peptide–polylysine conjugates into complexes, where only half of the plasmid DNA is complexed with transferrin–polylysine [TfpL(4μg)+Influ1pL], resulted in an over 100-fold improvement over TfpL–DNA complexes alone. When the DNA was first completely saturated with transferrin conjugates [TfpL(8μg)+Influ1pL], the additional free lysogenic conjugate (which cannot bind the DNA) still had some effect, but a much lower one than if the peptide was part of the complex. Free peptides 1 and 2 had no promoting effect on gene transfer, but clearly showed toxic effects at higher concentrations. In the special case of HeLa cells, unmodified polylysine exerted a considerable, but less pronounced, enhancement of the transferrin–polylysine-mediated gene transfer than the peptide–polylysine conjugates (see Fig. 6). This unspecific polylysine effect in HeLa cells was blocked by raising the serum content of the medium to 10–20% (data not shown).

Different combination complexes consisting of DNA, transferrin–polylysine, and optimum amounts of the peptide conjugate Influ1pL or one of the three Influ2pL conjugates with different peptide-to-polylysine ratios were tested for gene transfer efficiency to the murine hepatocytic cell line BNL CL.2 (Fig. 4*A*). A clear correlation between the content of peptide in the conjugate and the gene transfer augmenting activity was observed. Combination complexes containing conjugates with a high molar ratio of membrane-disruptive peptide to polylysine [Influ2pL(8:1) or Influ1pL(4:1)] produced up to a 1000-fold increase in luciferase gene expression. The use of conjugates with a low content of peptide [Influ2pL(1:1)], of unmodified polylysine, or of Influ3pL (data not shown) was far less effective in these cells. These findings also correlate with the leakage activities of the conjugates in the liposome assay (Fig. 4*B*). The conjugates of influenza peptides 1 and 2 showed comparable augmentation of the gene transfer to hepatocytes.

A comparison of several transferrin–polylysine-mediated gene transfer procedures is shown in Fig. 5. The standard transferrinfection to BNL CL.2 cells using DNA–transferrin–polylysine complexes resulted in limited gene expression in both the absence and presence of chloroquine (Fig. 5). Inclusion of the peptide conjugate Influ1pL considerably

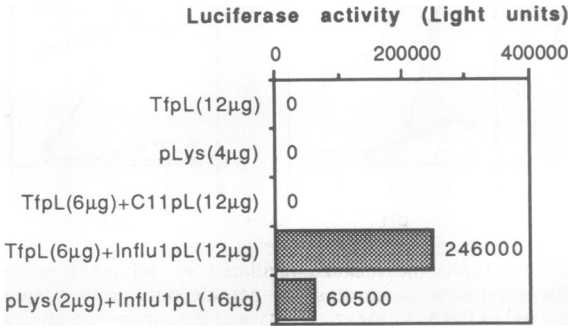

FIG. 2. Transfection of K562 cells. Complexes of 6 μg of pCMVL DNA and the indicated amounts of TfpL190B conjugate (TfpL) [or $pLys_{300}$ (pLys)] Influ1pL, or C11pL were added to K562 cells (see *Materials and Methods*). The cells were incubated at 37°C for 24 hr and then harvested for the luciferase assay. In this figure and Figs. 3–5, the values shown represent the total luciferase activity of the transfected cells.

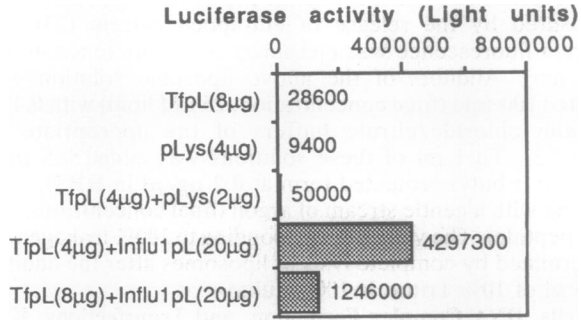

FIG. 3. Transfection of HeLa cells. Complexes containing 6 μg of pCMVL DNA and the indicated amounts of the TfpL290 conjugate (TfpL) [or $pLys_{300}$ (pLys)] and Influ1pL conjugate were prepared and added to the cells as described in *Materials and Methods*. The cells were incubated at 37°C for 2 hr, then 2.5 ml of medium containing 10% FCS was added. After 24 hr the cells were harvested for the luciferase assay.

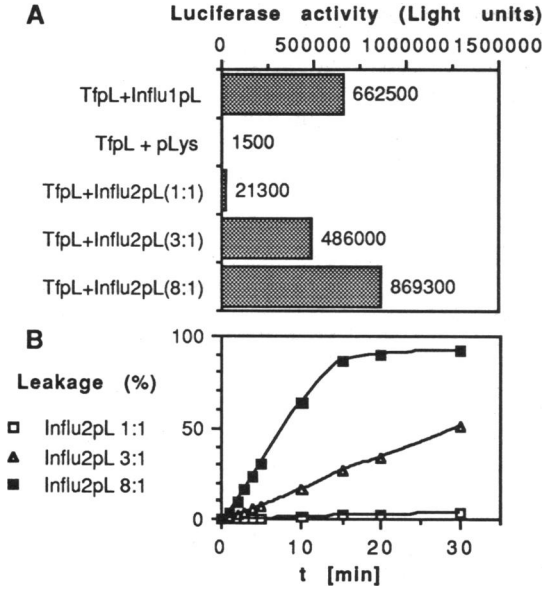

FIG. 4. Transfection efficiencies of different influenza peptide conjugates. (*A*) Complexes of 6 μg of pCMVL DNA, 4 μg of TfpL290 conjugate (TfpL), and 20 μg of Influ2pL conjugates with molar ratios of peptide to polylysine of 1:1, 3:1, and 8:1 or, for comparison, 20 μg of $pLys_{300}$ (pLys) or 20 μg of conjugate Influ1pL were added to BNL CL.2 cells (as described in *Materials and Methods*). The cells were incubated at 37°C for 4 hr, then 2 ml of medium containing 18% FCS was added. After 24 hr, the cells were harvested for the luciferase assay. (*B*) Liposome leakage activity of Influ2pL conjugates (equivalent to 2.5 μg of polylysine) at pH 5.0, performed as described in *Materials and Methods*.

increased gene expression. In combination with chloroquine, the gene expression was further enhanced (see Discussion).

β-Galactosidase Gene Delivery to HeLa Cells and Detection of Expression at the Cellular Level. DNA complexes containing a β-galactosidase reporter gene were used for delivery to HeLa cells, and the subsequent gene expression was visualized on the cellular level (Fig. 6). Using transferrin–polylysine-complexed DNA, only a few cells (≈1 in 1000) expressed β-galactosidase (data not shown). Combination complexes, additionally including the influenza peptide conjugate Influ1pL, showed a strong expression of β-galactosidase in 3–10% of the cells (Fig. 6*A*). The control experiment with complexes containing transferrin–polylysine conjugates together with comparably high amounts of unmodified polylysine resulted in expression in ≈0.5% of the cells (see Fig. 6*B*). Cells transfected with the combination complexes replicated normally and transferred the introduced gene equally to their daughter cells, leading to groups of predominantly four expressing cells 48 hr after transfection, which correlates with a progression through two cell cycles.

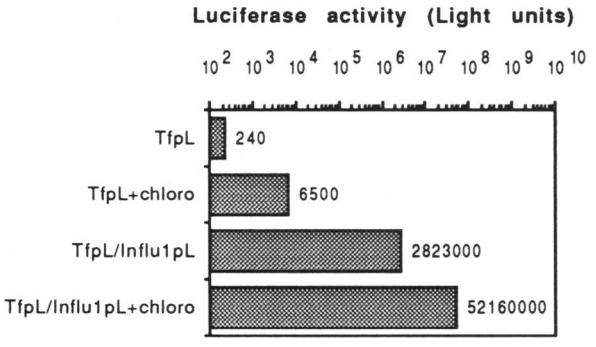

FIG. 5. Transfection of murine hepatocytes. The effect of chloroquine (chloro), Influ1pL, or the combination of both agents on transferrin–polylysine (TfpL)-mediated luciferase gene delivery (6 μg of pCMVL plasmid, 24 hr) to BNL CL.2 hepatocytes is shown.

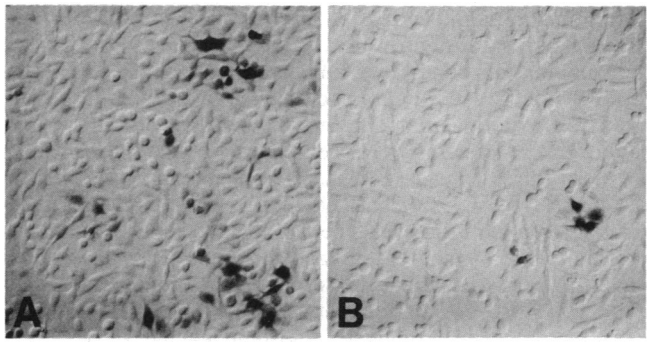

FIG. 6. *In situ* demonstration of β-galactosidase expression in HeLa cells. Transfections using pCMV–β-gal DNA (28) complexes were carried out as described in Fig. 3. After 48 hr, staining reactions were performed for 1 hr (see *Materials and Methods*). (*A*) HeLa cells transfected with 6 μg of pCMV–β-gal complexed with 4 μg of TfpL290 and 20 μg of Influ1pL. Approximately 3–10% of the cells show a strongly positive reaction for β-galactosidase. (*B*) HeLa cells transfected with 6 μg of pCMV–β-gal complexed with 4 μg of TfpL290 and 10 μg of polylysine. (β-galactosidase expression is observed in ≈0.5% of the cells.)

DISCUSSION

When complexes of the transferrin–polylysine conjugate (12, 17) and DNA are incubated with cells, the complexes bind to the transferrin receptors and are internalized by the cells, leading to expression of genes contained within the DNA (13, 29). In order to obtain high levels of gene expression, measures have to be taken to protect the DNA from degradation in lysosomes and/or release it from intracellular vesicles into the cytoplasmic compartment and finally into the nucleus of the cell (15). The approach described here makes use of the efficient mechanisms that many viruses (for instance, adenovirus and influenza virus) employ for the release from endosomes. Instead of using whole free viruses, we have taken synthetic fusogenic peptides derived from an influenza virus envelope protein and linked them to a complex containing DNA, transferrin, and polylysine, with the hope that the activity, which in its natural context causes fusion with the endosomal membrane, may disrupt endosomes in the context of our artificial complexes. In the case of influenza virus, the fusion activity has been extensively studied (30). The factor responsible for fusion of viral and endosomal membranes is the viral membrane glycoprotein hemagglutinin, a homotrimer, each monomer of which consists of two polypeptides, HA-1 and HA-2, connected through a disulfide bond (31). The membrane fusion activity is triggered by an acid-induced change, which exposes the highly conserved hydrophobic N-terminal region of the HA-2 subunit, allowing it to interact with the endosomal membrane. Synthetic peptides corresponding to the N terminus of influenza HA-2 are able to fuse artificial lipid membranes and cause leakage of aqueous liposomal contents (27, 32). Such peptides were shown to increase their α-helicity when interacting with lipid membranes (27, 32). Protonation of the acidic residues (glutamic acid at positions 11 and 15 and aspartic acid at position 19, which align on a predicted α-helix and exert charge repulsions at neutral pH) is expected to promote transition to a helical structure concomitant with membrane binding and destabilization. Peptides containing the sequences of the N terminus of HA-2 and polylysine conjugates thereof show pH-dependent leakage activities in a liposome assay (Fig. 1). Peptide 1 has leakage activity only at low pH. This is consistent with the model that the

additional glutamic acid at position 4 would further destabilize an α-helix at neutral pH.

Combination complexes consisting of plasmid DNA encoding luciferase or β-galactosidase as reporter genes, transferrin–polylysine, and membrane-disruptive peptide–polylysine conjugates were used for gene transfer to cell lines of the erythroid lineage (K562 cells) or epithelial lineage (HeLa cells and BNL CL.2 hepatocytes) as well as NIH 3T3 fibroblasts (data not shown). The influenza peptide conjugates considerably augmented the transferrin–polylysine-mediated gene delivery. By using different peptide–polylysine conjugates, a close correlation between the content of peptide within a polylysine conjugate, liposome leakage activity, and augmentation of gene expression mediated by a particular peptide conjugate was found (Fig. 4). The polylysine conjugates of the two different peptide sequences show no significant differences in the gene transfer-enhancing activities.

Inclusion of chloroquine shows a promoting effect on the gene-transfer complexes, suggesting that the entry occurs via an endocytic pathway (Fig. 5). The synergistic effect of chloroquine (which raises the pH of internal vesicles and therefore should reduce the peptide's pH-dependent leakage activity) was unexpected but can be explained by a protection of the conjugates from lysosomal degradation, by osmotic destabilization of the endocytic vesicles by internal accumulation of chloroquine (25, 33), and/or by the observation that the influenza peptide conjugates display some residual leakage activity at neutral pH.

Expression of a galactosidase reporter gene in HeLa cells (Fig. 6) demonstrates that the influenza peptide conjugates facilitate transferrinfection up to a level of high expression in about 5–10% of all cells. This frequency is low compared to the transferrinfection performed in the presence of free or DNA-bound defective adenoviruses (34, 35) where frequencies of ≥90% are observed. However, with the use of whole virus, some cytopathic effects on the transfected cell population can be observed (34). With the DNA complexes containing the membrane-disruptive peptides, no toxic effects have been found.

Our transport complexes can be likened to artificial viruses that mimic the viral transfer of genes into cells but are devoid of all cytopathic and replicative functions of natural viruses. Once higher efficiencies of gene transfer have been obtained, such artificial viral complexes may become the method of choice. The present report may be considered as a first step toward this goal. The model complexes described here contain amphipathic peptides as the minimal functional unit for specific membrane disruption but lack the ordered architecture of viral surfaces thought to mediate optimal entry.

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