Enhanced *In Vitro* Oligonucleotide and Plasmid DNA Transport by VP1 Virus-Like Particles

S. Henke,[1,2] A. Rohmann,[1,2] W.M. Bertling,[3] Th. Dingermann,[2] and A. Zimmer[1,4]

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Purpose. We developed a prokaryotic expression system to express the major capsid protein of Polyomavirus, VP1. Furthermore, we investigated the transport of single stranded (ss) and double stranded (ds) DNA, mediated through VP1 as drug delivery system into mouse fibroblasts.

Methods. To study DNA delivery we used two kinds of DNA, a ssDNA fragment (19mer) and dsDNA (plasmid pEGFPN1, 4.7 kb or a FITC-labelled dsDNA fragment, 1.8 kb).

Results. The uptake of VP1 capsoids loaded with FITC-labelled oligodeoxynucleotides (FODNs) was observed. VP1 pentamers loaded with condensates of dendrimer/dsDNA fragments (FITC-labelled) resulted in significantly higher fluorescence signal in the cytoplasm of NIH 3T3 cells in comparison to control experiments without VP1. Additionally, VP1 capsoids loaded with plasmid pEGFPN1 without dendrimers resulted in an approximately 10 fold higher transfection rate in comparison to blank DNA controls.

Conclusions. Our results demonstrated the potential of VP1 capsoids as DNA delivery system. EGFP expression was significantly enhanced when plasmid DNA was delivered via VP1 capsoids, compared to control experiments with naked DNA.

KEY WORDS: polyoma virus; VP1 capsoids; NIH 3T3 cells; DNA uptake; EGFP; plasmid transfection.

INTRODUCTION

Polyomavirus forms a nonenveloped capsid organised as a double shell of three different proteins. The outer shell of the virion is composed of 72 pentamers of the major capsid protein VP1 in an icosahedral surface lattice (1). Each pentamer is connected with an internal minor protein VP2 and VP3, which together form the inner shell of the polyoma virus capsid (2). The inner core contains the double stranded circular DNA genome of 5.29 kb, which is associated with host histones (3,4).

VP1 acts as a major ligand for certain membrane receptors (= receptor-mediated endocytosis) during virus infection (5). Furthermore, the N-terminus of the VP1 protein contains a DNA-binding domain (6). Combining these aspects, VP1 proteins provide a targeting as well as a drug binding site when used as drug carriers for gene therapy.

Early transfection studies of babymouse kidney cells with native polyomavirus produced various types of virus particles, i.e. pseudovirions containing host-DNA with a heterogeneous molecular mass of 1-3 $\times 10^6$ Da. These particles were used for gene transfer in human and mouse embryonic cells (7).

In later investigations polyoma-like-particles (PLPs) prepared from empty Polyoma capsoids and carrying recombinant DNA were used. An efficient transport of supercoiled polyoma DNA, ssDNA, rRNA and synthetic homopolymers in rat cells was shown by Slilaty et al. (1982) (8).

Our group tested the transfection of ds/ssDNA and DNA-protein complexes into the nuclei of mammalian cells (9). Polyoma capsids isolated from mice showed a higher DNA transfection efficiency than an alternatively applied liposome system (10). These PLPs were also applied to other prokaryotic and eukaryotic expression systems in *E. coli, Baculoviral systems* and *S. cerevisiae* (11). Nevertheless, contamination of empty viral capsoids with full infectious virions or pseudovirions loaded with host DNA was reported for PLPs isolated from mice or insect cells (12,13).

In order to avoid host cell DNA impurities, further research focussed on VP1 proteins as DNA drug delivery systems. After expression of the major structural protein VP1 in *E. coli,* Salunke et al. (1986) showed an assembly to capsid-like particles (2). These particles were stabilised by Ca^{2+}-ions and intrapentameric disulphide-bonds (14).

Additionally, Forstova et al. (1993) reported a self-assembly of VP1 capsoids in Sf9-cells and showed a coexpression of VP1, VP2 and VP3 (15). DNA-packaging with support of host histones was limited to a size of 5 kb, similar to the polyoma virus genome (12).

The main focus of our study was to avoid all these restrictions and to simplify the preparation of a protein carrier we developed an unique prokaryotic expression system for a His-tagged VP1 fusion protein. This protein was used to generate polyoma virus-like particles consisting only of the recombinant VP1 without host DNA contamination (16).

Our investigations demonstrate that VP1 capsoids can be used as a transfection agent for various DNA molecules such as plasmid DNA and smaller DNA constructs like antisense oligodeoxynucleotides. In addition, a novel DNA loading strategy based on dendrimer technology is introduced.

MATERIALS AND METHODS

Prokaryotic Expression System

VP1 was expressed in *E. coli* RB791 (W3110, *lacI^q*, L8) transformed with plasmid pHB17/6 carrying the coding sequence of VP1, as described earlier (16,17).

Protein Purification and Capsoid Assembly *In Vitro*

For cell lysis the bacteria culture were suspended in cold lysis buffer containing lysozyme (2 mg/ml, Sigma-Aldrich Chemie GmbH, Deisenhofen, Germany) and incubated on ice for 30 min, following sonication on ice until the lysate lost its viscosity. Cell debris was sedimented and the supernatant was mixed with Ni-Nitrilotriacetate-agarose (Ni-NTA-agrose, Qiagen GmbH, Hilden, Germany). After overnight incubation at 4°C VP1 was purified by factor Xa cleavage (1 μg/μl,

[1] Institute for Pharmaceutical Technology, Biocenter—Johann Wolfgang Goethe-University, Frankfurt am Main, Germany.
[2] Institute for Pharmaceutical Biology, Marie-Curie-Str. 9, 60439 Frankfurt am Main, Germany.
[3] november AG, Gesellschaft für Molekulare Medizin, Ulrich-Schalk- Str. 3, 91056 Erlangen, Germany.
[4] To whom correspondence should be addressed. (e-mail: Zimmer@em.uni-frankfurt.de)

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NEB, Beverly, USA) in order to remove the N-terminal 6 ×His affinity tag (16). The VP1 protein appears as a pentamer, which is stable under reducing and chelating conditions (10 mM Tris/HCl; 150 mM NaCl; 5% (v/v) glycerol; 1 mM EDTA; 15 mM 2-mercaptoethanol, pH 7.2) (18). VP1 capsoids were obtained by dialysis under high ionic strength conditions (10 mM Tris/HCl; 150 mM NaCl; 5% (v/v) glycerol; 750 mM $(NH_4)_2SO_4$, pH 7.2).

Electron Microscopy

A protein aliquot (5 μl) was placed on a pioloform-coated copper/paladium grid (200 mesh, Plano, Wetzlar, Germany). Samples were stained with 2% (w/v) uranyl acetate solution (Merck, Darmstadt, Germany) for 15 s and protein structures were visualised with a Zeiss CEM 902 transmission electron microscopy operating at 80 kV.

Particle Size Measurement by PCS

Particle diameters were determined by photon correlation spectroscopy (PCS, Brookhaven Instruments Corp., Holtsville, NY). The measurements were carried out at a scattering angle of 90° and a temperature of 25°C. The count rate was adjusted to 20–30 kHz by diluting each sample with filtered HBS-buffer (21 mM HEPES; 137 mM NaCl; 5 mM KCl; 0.7 mM Na_2PO_4; pH 7.4) (0.22 μm cellulose nitrate filter, Schleicher&Schuell, Dassel, Germany).

Oligonucleotide Packaging

VP1 capsoids were loaded with oligodeoxynucleotides (ODNs) (19mer; ACG TTC CTC CTG CGG GAA G; MWG-Biotech AG, Ebersberg, Germany) in loading buffer (10 mM sodium acetate; 150 mM NaCl; 5% (v/v) glycerol, pH 5) by osmotic shock (19) and loading capacity was measured by HPLC as described earlier (16). The buffer was adjusted to isotonic conditions by addition of 5% mannitol.

Plasmid DNA Packaging

The 4733 bp mammalian expression vector pEGFPN1 containing the enhanced green fluorescence protein (EGFP) coding sequence under control of the CMV promotor (CLONTECH Laboratories GmbH, Heidelberg, Germany) was used to study VP1 packaging of larger DNA constructs. DNA packaging was performed by dialysis of VP1 pentamers under high ionic strength conditions (protein/DNA ratio 5:1; w/w).

DNA Condensation with Histone Sulphate

A calf thymus histone sulphate mixture containing histones H1; H2A; H2B; H3 and H4 (Fluka AG, Buchs, Switzerland) was used. Different histone sulphate/DNA ratios (0.5:1–5:1; w/w) were mixed at room temperature for 1 hour in HBS-buffer. The resulting histone sulphate/DNA complexes were dialysed in the presence of VP1 pentamers in various ratios (30:1; 3:1; 0.3:1; w/w; absolute protein amount: 1 μg/well) under high ionic strength conditions. Prior to transfection the formed capsoids were dialysed against HBS-buffer.

DNA Condensation with Dendrimers

Condensation of activated dendrimers (3 μg/μl, SuperFect, QIAGEN GmbH, Hilden, Germany) with DNA at different ratios (15:1; 6:1; 3:1; w/w) were prepared according to the manufacturer's protocol from Qiagen. The complexes were dialysed in the presence of VP1 pentamers (total protein amount: 25 μg/well) under high ionic strength conditions.

Cellular Uptake of FITC-Labelled Oligonucleotides

NIH 3T3 mouse fibroblasts were grown in Dulbecco's modified Eagle's medium (DMEM; GIBCO/BRL, Eggenstein, Germany) supplemented with 10% fetal calf serum (FCS, Biochrom KG, Berlin, Germany). Transfection of 0.2×10^5 cells/ml each was performed in 8-well chamber slides (0.8 cm^2; 400 μl; Nunc, Roskilde, Denmark). Capsoids were loaded with FITC-labelled oligodeoxynucleotides (FODNs) at a protein/DNA weight ratio of 6:1 as described. The cells were incubated for 1 hour with loaded capsoids. The medium was changed and cells were incubated for 3 hours in fresh culture medium. Cell membranes were stained with TRITC-conjugated concanavalin A (Sigma-Aldrich Chemie GmbH, Deisenhofen, Germany) for 1-3 min and fixed with absolute methanol for 7 min at –20°C. The samples were embedded in 10% Mowiol 4-88 (Hoechst AG, Frankfurt, Germany) and the DNA uptake was investigated by confocal laser scanning microscopy (CLSM, Leica, Wetzlar, Germany, Imaris Software, Bitplane AG Zürich, Switzerland).

Cellular Uptake of FITC-Labelled dsDNA Fragment

A dsDNA fragment (1780 bp) was labelled with a PCR fluorescein labelling mixture (Boehringer Mannheim, Mannheim, Germany). The dendrimer/DNA complexes (15:1; 6:1; 3:1; w/w) were prepared as described. The preparations were dialysed in the presence of VP1 pentamers (absolute protein amount: 25 μg/well) under high ionic strength conditions. Alternatively, these complexes were incubated with VP1 pentamers (total protein amount: 25 μg/well) under reducing and chelating conditions for 20 min at 37°C. NIH 3T3 cells were cultivated in 8-well chamber slides (0.2×10^5 cells/ml). Two days later cells were transfected as described above.

Cell membranes were counterstained with Alexa^{594} – concanavalin A (Molecular Probes Europe BV, Leiden, Netherlands). Following fixation with 5% paraformaldehyde/PBS solution, cells were embedded in Mowiol 4-88. The uptake of fluorescent DNA fragments was observed by CLSM.

Plasmid DNA Transfection with VP1

NIH 3T3 mouse fibroblasts were transfected with pEGFPN1-loaded VP1 capsoids (total protein amount: 25 μg/well). After 48–72 hours of cultivation in growth medium, expression of enhanced green fluorescence protein (EGFP) in living cells was investigated by fluorescence microscopy.

Immunofluorescence Assay

Cells were incubated with VP1 capsoids, washed with PBS fixed with 5% paraformaldehyde/PBS solution and permeabilised with 1% Triton/PBS solution. After blocking with 1% BSA, cells were washed with PBS and incubated for 60 min at 37°C with an anti-VP1 (mouse) monoclonal IgG1

(kindly provided by Dr. M. Pawlita, DKFZ, Heidelberg, Germany). All preparations were washed five times with PBS and followed by an incubation with a fluorescein-conjugated goat anti-mouse IgG (ICN Biomedicals GmbH, Eschwege, Germany) for 45 min at 37°C, embedded in Mowiol 4-88. Image analysis was performed by CLSM.

RESULTS

Protein Expression, Isolation and Assembly *in vitro*

Protein expression was induced with 100 μM IPTG in the logarithmic growth state of bacteria. Protein purification was achieved with a Ni-NTA-agarose followed by factor Xa cleavage. The protein purity was determined by SDS-PAGE and VP1 was detected with the expected size of 42.5 kDa (Fig. 1). VP1 was taken from the soluble protein fraction with a quantity of about 1.5-1.8 mg protein/l culture volume. As determined by transmission electron microscopy and photon correlation spectroscopy, the protein was isolated as a homopentamer of approx. 8 nm in diameter (Fig. 2A) and was capable of assembly into capsoids ranging between 24 and 50 nm in diameter (Fig. 2B).

Oligonucleotide Packaging

Most efficient oligodeoxynucleotide (ODN) packaging of VP1 capsoids was obtained by osmotic shock treatment at a protein/DNA weight ratio between 6:1 and 6:0.5. ODN packaging achieved in loading buffer (pH 5.0) resulted in mean values of about 38% (± 5%). The adsorption of ODNs

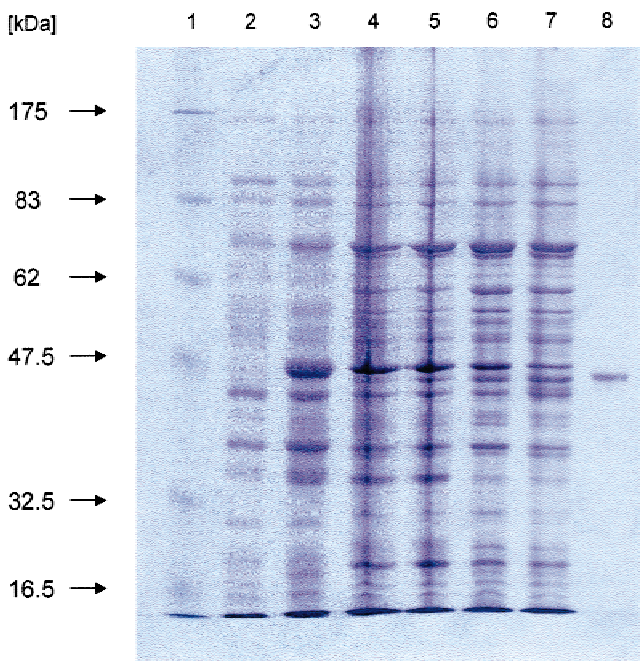

Fig. 1. SDS-PAGE: Lane 1: MW Standard, Lane 2: Sample prior induction, Lane 3: Sample 6 hours after induction with 100 μM IPTG, Lane 4: Sample after lysozyme treatment, Lane 5: Sample after ultrasonification of the bacteria suspension, Lane 6: Protein sample after centrifugation at 10.000 xg, Lane 7: Protein sample after incubation with Ni-NTA-agarose, Lane 8: Purified protein fraction after factor Xa cleavage.

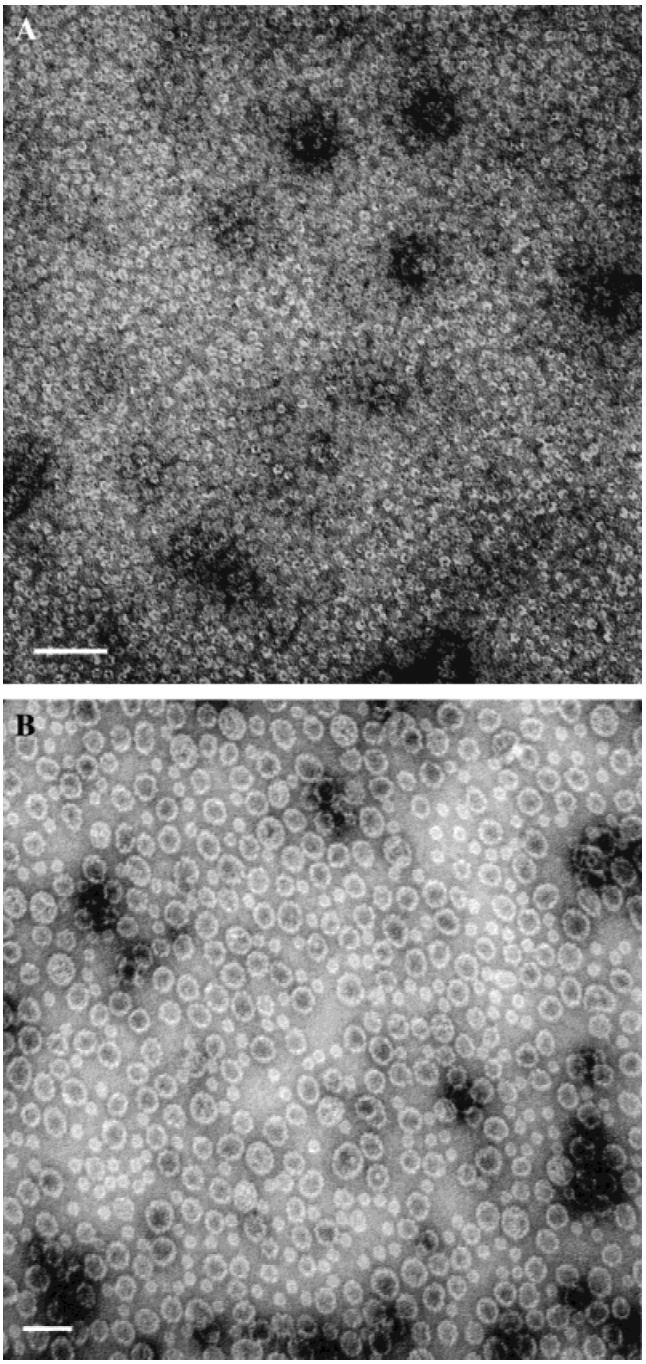

Fig. 2. (A) TEM-Picture of VP1 pentamers under reducing and chelating conditions (scale bar: 50 nm). (B) TEM-Picture of VP1 capsoids after dialysis under high ionic strength conditions (scale bar: 82 nm).

to VP1 protein was found to be salt and pH dependent: Less than 2% ODNs were adsorbed to the VP1 capsoids after incubation with 1 M NaCl. Loading after osmotic shock at pH 7.2 was also negligible.

Plasmid DNA Condensation and Packaging

The highest loading efficiency for plasmid DNA was achieved by osmotic shock treatment at an equimolar VP1/

DNA ratio. We observed mean values of about 52% DNA loaded to VP1 capsoids. Only DNA fragments of 1.5 kb–1.8 kb were protected against nuclease digestion following this protocol (16).

In this study we investigated two new packaging strategies based on DNA condensation prior to DNA loading of VP1 proteins in order to facilitate packaging of larger DNA constructs.

One strategy was to condensate the plasmid DNA with histone sulphate mixture. Successful *in vitro* DNA condensation was demonstrated by a gel shift assay (Fig. 3A). DNA migration decreased with increasing histone concentrations (histone/DNA ratios between 0.5:1 and 5:1) (Fig 3A: lane 7-9). The resulting histone/DNA complexes were further characterised by particle size measurements using photon correlation spectroscopy (Table 1). In general, histone/DNA aggregates showed a very large size distribution from approximately 500 nm to 6200 nm. Since packaging of such large aggregates with 50 nm VP1 capsoids is impossible, a different condensation method based on activated dendrimer technology was investigated.

Activated dendrimers are able to condense DNA by strong interactions of negatively charged phosphate groups of the DNA with positively charged amino groups of the dendrimer molecule. A gel shift assay of dendrimer/DNA complexes showed that mass ratios ranging from 3:1 up to 15:1 (dendrimer:DNA, w/w) were able to condense the complete DNA (Fig. 3B).

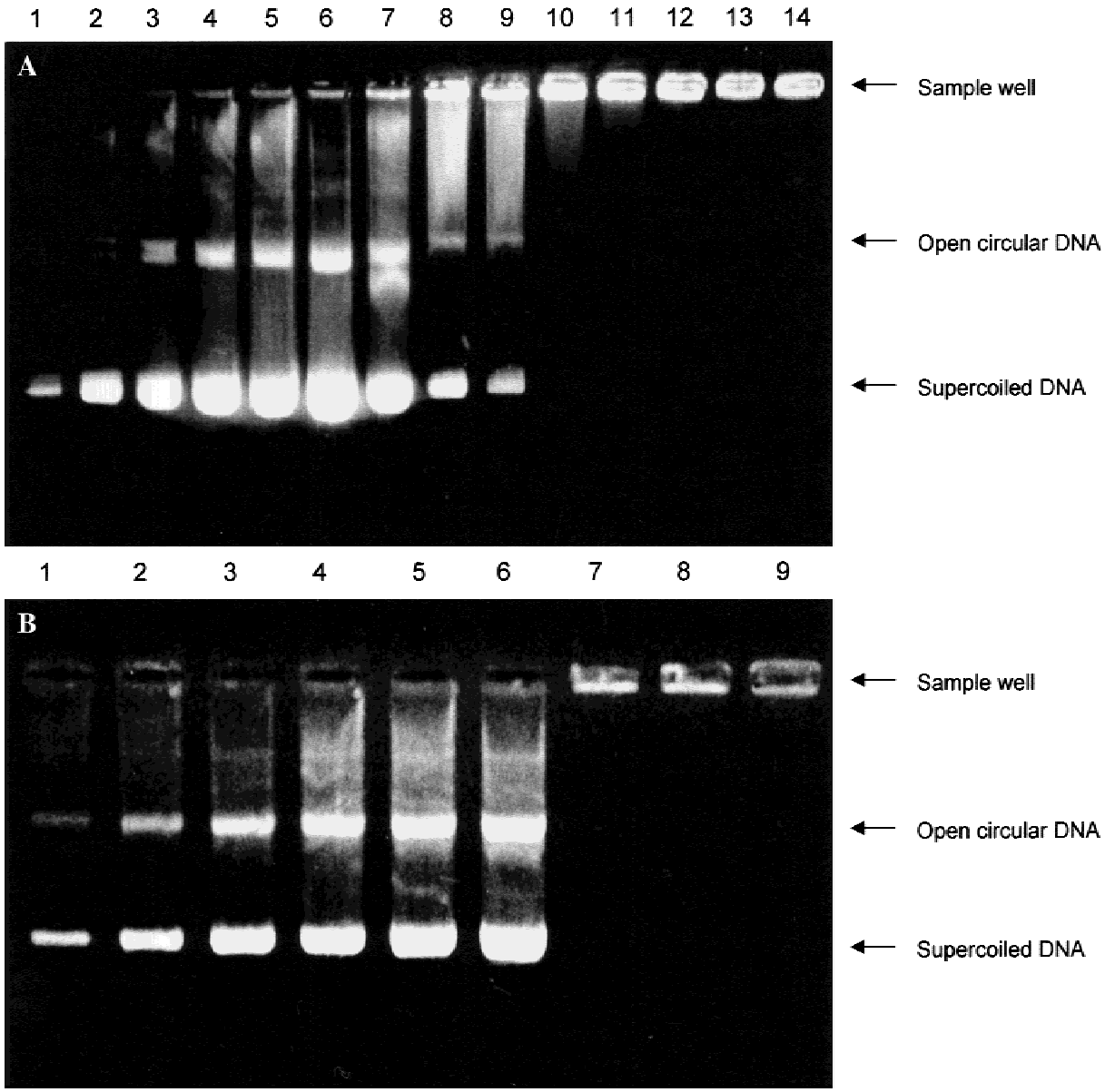

Fig. 3. (A) Gel shift assay with histone sulphate/DNA complexation (w/w): Lane 1-6: circular plasmid DNA standards ranging from 1-11 μg. Lane 7: 0.5:1 ratio, 37% DNA complexation; Lane 8: 1:1 ratio, 80% DNA complexation; Lane 9: 1.5:1 ratio, 95% DNA complexation; Lane 10-14: 2:1, 2.5:1, 3:1, 4:1, 5:1 ratio, total DNA immobilisation. (B) Gel shift assay with dendrimer/DNA complexation (w/w): Lane 1-6: circular plasmid DNA standards ranging from 0.1-1 μg; Lane 7-9: 3:1, 6:1, 15:1 ratio, total DNA immobilisation.

Table 1. Mean Particle Size of Different Histone/DNA Condensates Determined by PCS

Histone sulphate/DNA condensates (w/w)	Effective diameter (nm)
Preparations in HBS-buffer	
30:1	507
3:1	n.d.[a]
0.3:1	3,044
Preparations in 0.4 M NaCl	
30:1	829
3:1	2,193
0.3:1	3,149
Preparations in 10% DMSO	
30:1	600
3:1	6,246
0.3:1	n.d.[a]

[a] Mean particle size above PCS detection limit.

FITC-Labelled dsDNA Fragment

Two DNA packaging methods were used in combination with VP1 proteins to deliver the dendrimer/DNA complexes. First, adsorption of only VP1 pentamers to the DNA preparation resulted in protein/DNA complexes where it is conceivable, that the protein was bound mainly by unspecific ionic interactions on the surface of the dendrimer/DNA complexes. The second strategy was based on a specific assembly of VP1 pentamers to capsoids under high ionic strength conditions in the presence of labelled DNA fragments. Transmission electron microscopy (TEM) images of these preparations showed capsoid like structures containing the FITC-labelled dsDNA fragment of 1.8 kb (Fig. 4A), whereas capsoid formation with unlabelled plasmid DNA of 4.7 kb resulted in incomplete capsoid structures (Fig. 4B).

Plasmid DNA

Standard plasmid packaging was performed by dialysis of DNA/VP1 pentamers against 750 mM ammonium sulphate buffer. DNA loading was found to be stable after replacement of the high ionic buffer with PBS in order to establish physiological buffer conditions.

Cellular Uptake of FITC-Labelled Oligonucleotides

NIH 3T3 fibroblasts were used to study the cellular uptake of FITC-labelled ODNs (FODNs). Analysis of cellular uptake and intracellular distribution by CLSM showed, that VP1 capsoids loaded with FITC-labelled ODNs entered significantly eukaryotic cells. Furthermore, we detected a stringent fluorescence in the nucleus (Fig. 5A). No cellular uptake of FODNs was obtained from control experiments without VP1 carriers (Fig. 5B).

Cellular Uptake of FITC-Labelled dsDNA Fragment

Uptake of larger DNA constructs in NIH 3T3 fibroblasts was investigated with FITC-labelled dsDNA fragment. Transfection was successfully obtained with preparations from dendrimer/DNA condensates at 15:1 (w/w) with 25 μg VP1 pentamers per well. The incubation protocol followed the procedure applied for oligodeoxynucleotides. The highest efficiency was obtained for preparations of dendrimer/DNA complexes with adsorbed VP1 pentamers. The results are shown in Fig. 6A. Significantly higher fluorescence intensities inside the mouse fibroblasts were found with the VP1 pentamers (Fig. 6A) in comparison to blank dendrimer/DNA complexes. Blank dendrimer/DNA condensates accumulated on the surface of the cell membranes (Fig. 6B).

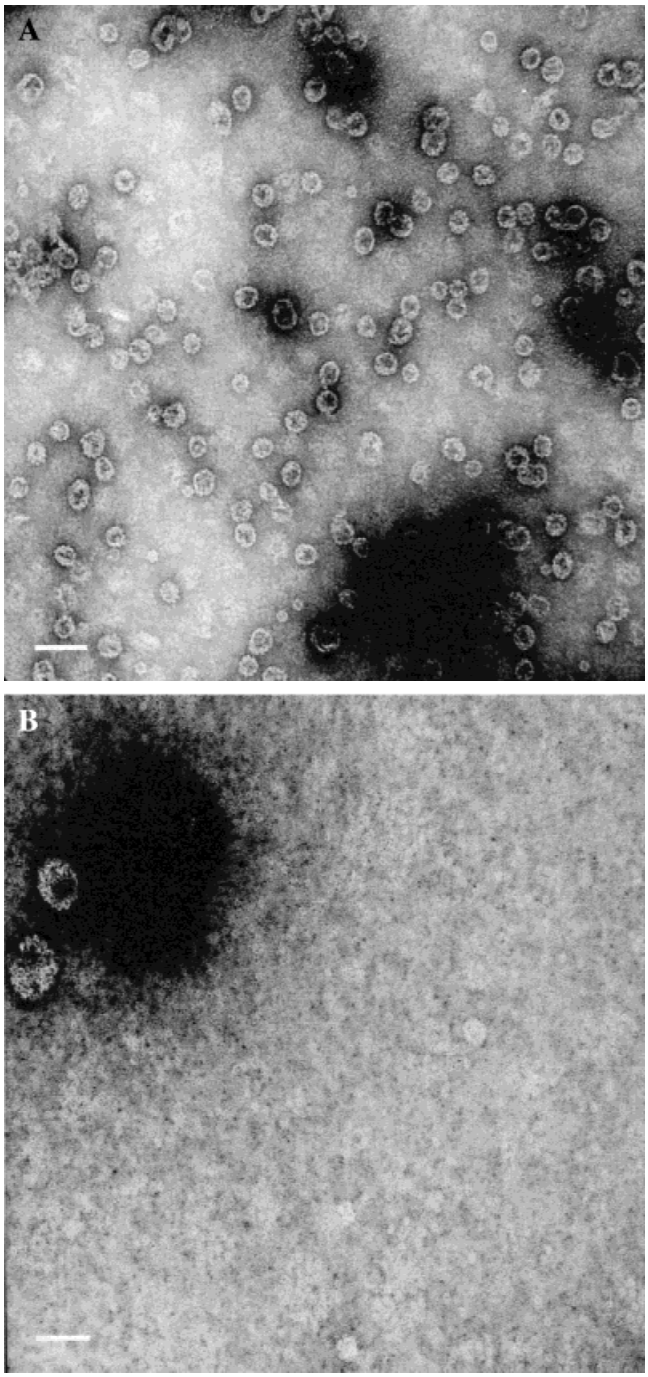

Fig. 4. TEM pictures of VP1 capsoids loaded with dendrimer/DNA condensates by dialysis under high ionic strength conditions. (A) FITC-labelled dsDNA (1.8 kb) (scale bar: 82 nm). (B) Plasmid DNA (4.7 kb) (scale bar: 50 nm).

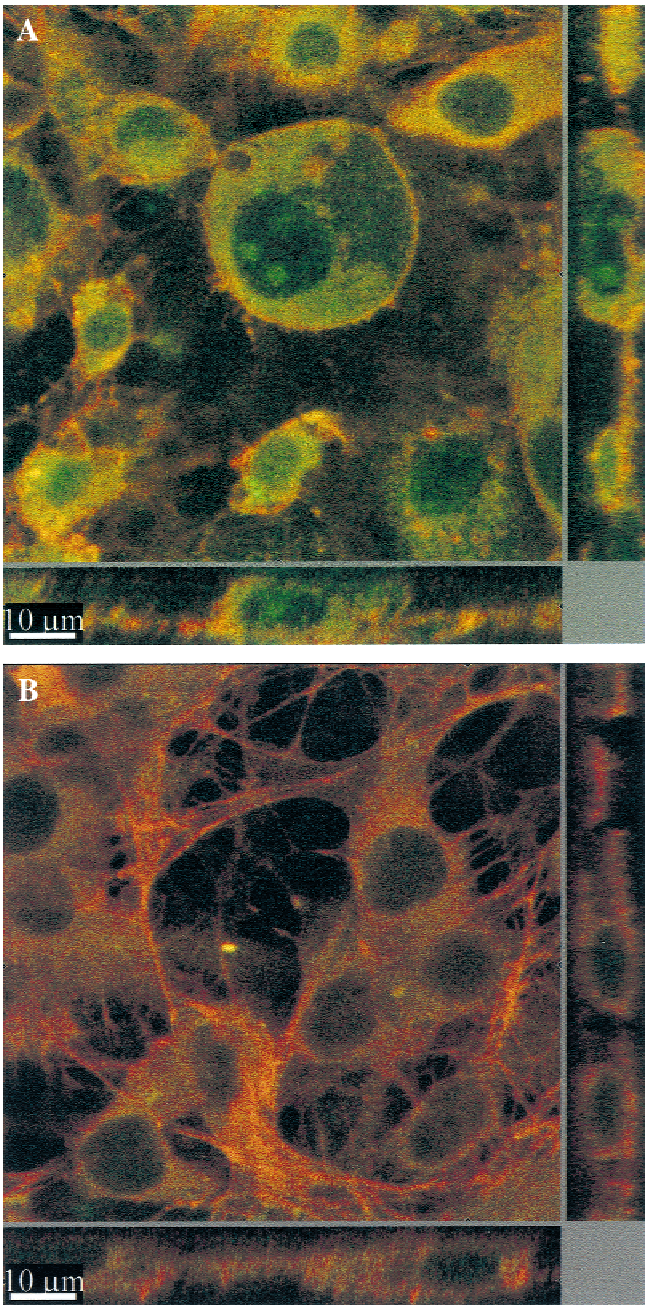

Fig. 5. Cellular uptake of capsoids loaded with FITC-labelled ODNs (FODNs) in NIH 3T3 cells. Cell membranes were counterstained with TRITC-labelled concanavalin A. (A) FODNs (1 µM) loaded to VP1 capsoids at a 1:6 ratio (w/w). (B) Control experiment FODNs without VP1 capsoids.

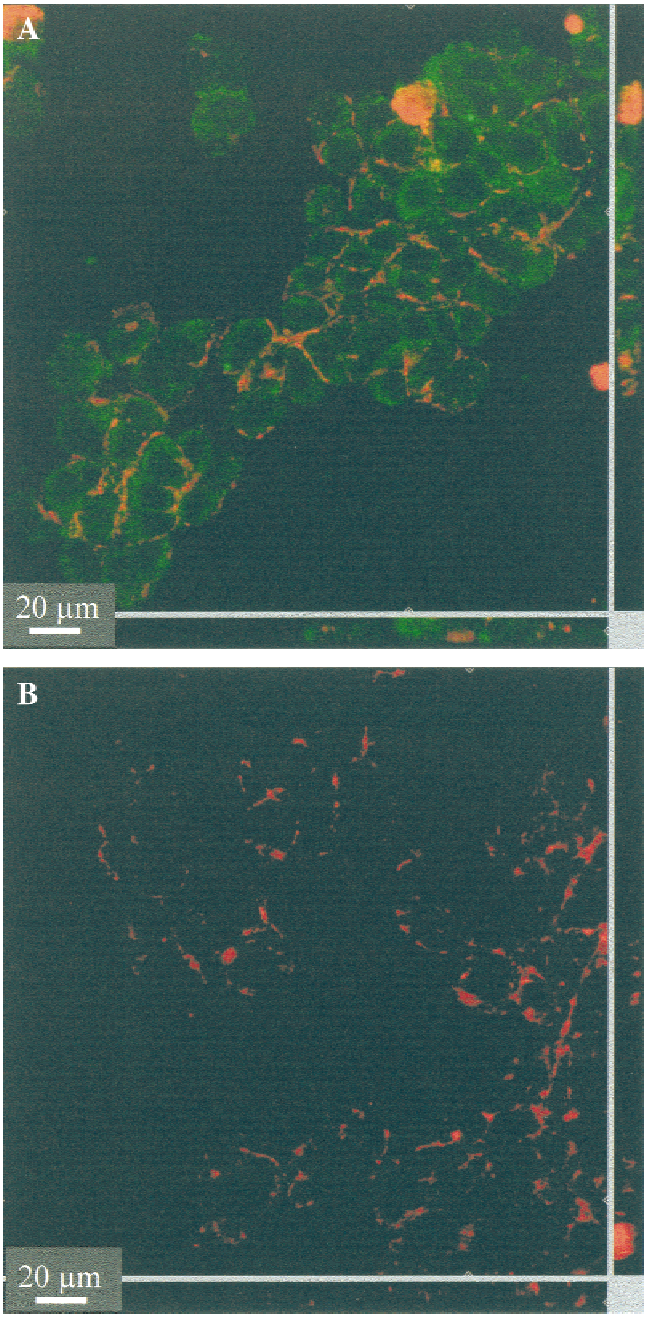

Fig. 6. Cellular uptake of dendrimer/FITC-labelled dsDNA (1.8 kb) condensates into NIH 3T3 cells. Cell membranes were counterstained with Alexa^{594} -labelled concanavalin A. (A) Preparation incubated with VP1 pentamers. (B) Control experiment without VP1 pentamers.

EGFP Plasmid DNA Transfection

VP1 pentamers dialysed against 750 mM ammonium sulphate in the presence of plasmid pEGFPN1 resulted in a 10 fold higher transfection rate compared to blank DNA controls. Successful EGFP transfection could be detected in approximately 10% of all cells whereas delivery of free plasmid DNA to NIH 3T3 cells without our VP1 delivery system was not detectable (Fig. 7). The uptake of dendrimer/DNA condensates with and without VP1 proteins resulted only in a negligible transfection enhancement (data not shown).

Immunofluorescence Assay

Uptake and subcellular localisation of VP1 capsoids in NIH 3T3 cells were analysed with an immunofluorescence assay. The cellular distribution and localisation of the VP1 proteins was found to be buffer dependent. Cells treated with VP1 proteins in 150 mM sodium chloride solution showed fluorescence signals in the cytoplasm as well as in the cell nucleus (Fig. 8B), confirming the results obtained with FODN uptake. Interestingly, cells incubated with a 150 mM sodium chloride solution adjusted to pH 7.4 showed a higher fluores-

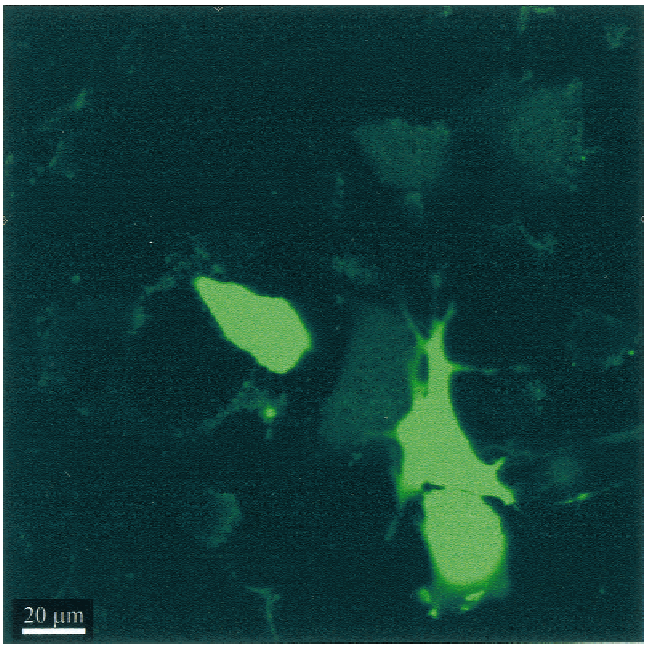

Fig. 7. EGFP expression in NIH 3T3 cells transfected with pEGFPN1/VP1 capsoids prepared by dialysis under high ionic strength conditions. The fluorescence microscopic picture was taken 48 hours after transfection.

cence signal in the cytoplasm, but no VP1 delivery into the nucleus (Fig. 8A).

DISCUSSION

A DNA drug delivery system was established using recombinant Polyoma virus core protein VP1 as pentameric subunits for an assembly of synthetic VP1 capsoids. Compared to standard transfection reagents like cationic lipids the main advantage for the application of an artificial virus shell is the natural principle of this DNA carrier system. Polyoma has a broad host range spectrum and facilitates a high transfection efficiency in many cell types by receptor-mediated endocytosis (20). In our study we demonstrated efficient cellular uptake of oligodeoxynucleotides as well as uptake of larger dsDNA fragments and efficient plasmid DNA transfection with recombinant VP1 capsoids.

Our previous work has shown that DNA packaging is restricted to dsDNA fragments of 1.5–1.8 kb. This is much less than polyoma virus shells cover naturally. The polyoma genome is about 5 kb in size.

For that reason we tested two different DNA condensation methods to facilitate packaging of larger double stranded DNA constructs and to optimise the transfection efficiency. The first method was based on histone/DNA complex formation. Histones are believed to play an integral role in the packaging of the viral DNA into the capsoids (4). In a recent study Gillock et al. (12) demonstrated packaging of DNA up to a size of 5 kb into polyoma-like particles, in which histone-like proteins were found. Based on these results we investigated the condensation of plasmid DNA with a calf thymus histone sulphate mixture of all five histones in different protein/DNA weight ratios. Only very large histone/DNA aggregates, resulted from a secondary aggregation processes. Similar results were obtained with purified calf thymus histone H4

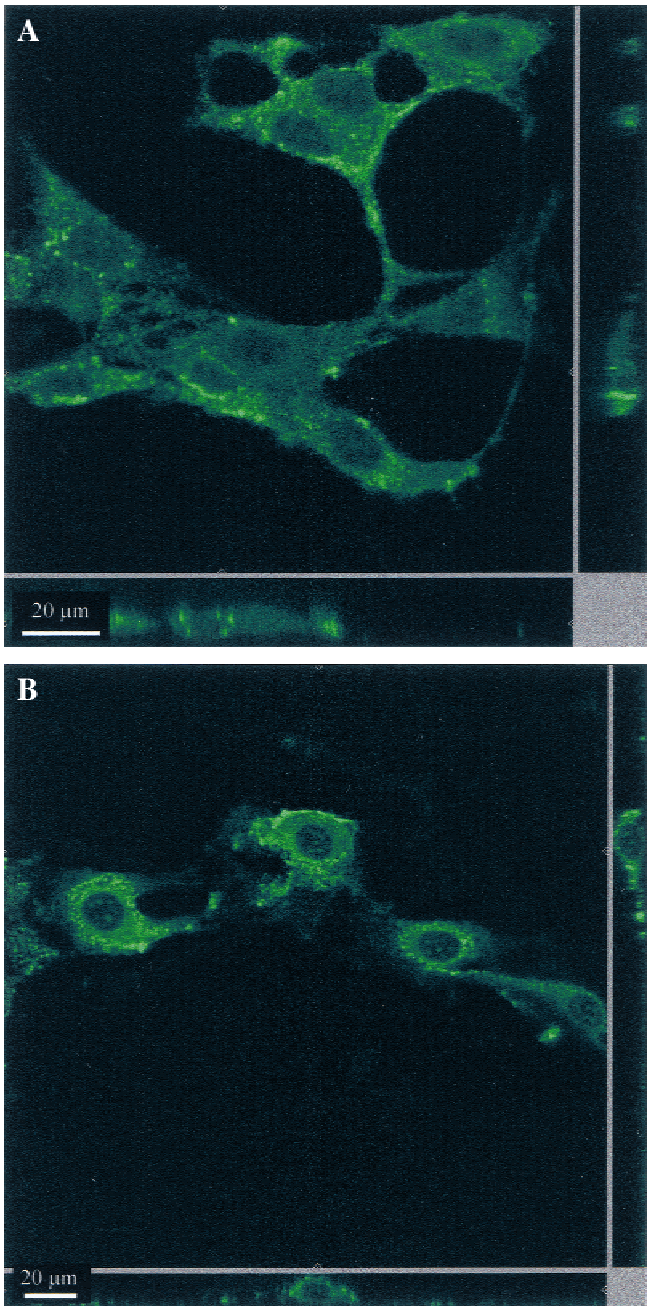

Fig. 8. Immunfluorescence staining of NIH 3T3 cells transfected with VP1 capsoids. (A) Transfection in PBS pH 7.4. (B) Transfection in loading buffer pH 5.

(data not shown). It is well known, that histones packaged DNA chromatins into very compact structures and it is conceivable, that highly condensed complexes between histones and DNA served as the starting point of this aggregation process. In a second strategy we made use of polycationic aminoamide dendrimers to condensate larger DNA molecules prior to packaging with VP1 proteins. These cationic dendrimers showed superior capabilities of DNA condensation resulting to smaller aggregates which are more suitable for a capsoid packaging than histone/DNA aggregates. In a recent study Tang et al. (21) determined the size distribution of dendrimer/plasmid DNA complexes by TEM. In these experiments a toroidal and spheroidal morphology with diam-

eters between 57 ± 16 nm and 70 ± 19 nm were observed. These complexes also protected DNA against serum- and intracellular nucleases (22). TEM pictures of FITC-labelled dsDNA fragments and plasmid DNA loaded to VP1 capsoids demonstrated that packaging under high ionic strength conditions was more inhibited by dendrimer/plasmid DNA condensates as by dendrimer/dsDNA fragment condensates. A homogenous capsoid formation was visualised with smaller dsDNA fragments complexed with dendrimers (see Fig 4A). Therefore, the DNA size has also a distinct influence on the VP1 assembly. At the moment the exact mechanism of the encapsulation of condensates into VP1 capsoids is unknown. Packaging mediated through unspecific interactions supported by the N-terminal DNA binding domain could be a possible explanation (6).

We tested uptake of such complexes in NIH 3T3 cells. These cells were chosen as *in vitro* test model because the polyoma virus naturally is a mouse virus and receptor binding sites were identified for polyoma-like particles on NIH 3T3 cells as N-linked glycoproteins (23). Furthermore, we observed no toxic effects with this cell line, mediated by the VP1 protein (data not shown). However, a decrease of the cell viability and growth rate were visualized after the transfection of dendrimer/DNA complexes alone or in combination with VP1 proteins into NIH 3T3 cells, due to the polycationic molecules (data not shown). These effects were found to be dose dependent. Immune response studies in the future must shown the influence of such dendrimer/DNA condensates *in vivo*.

The cellular uptake of FITC-labelled ODNs bound to VP1 capsoids by an osmotic loading technology resulted in a diffuse cytoplasmic distribution and a distinct intranuclear fluorescence (Fig. 5). This demonstrates the capability of our delivery system to transport oligodeoxynucleotides into the nucleus.

We further showed that VP1 proteins were effective as transfection enhancers for dendrimer/FITC-labelled dsDNA fragments. This was clearly shown by a higher intracellular fluorescence of these transfected cells in contrast to control cells treated only with dendrimer/DNA condensates without VP1 pentamers (Fig. 6).

In addition to cellular uptake experiments with the FITC-labelled dsDNA fragment transfection experiments were performed with EGFP plasmid DNA. As a positive control for this plasmid DNA we used the cationic liposome DOTAP as a carrier system. Toxic effects were observed during these experiments which were attributed to the cationic liposomes (data not shown). Therefore, a further comparison between DOTAP and VP1 proteins was not performed.

In a first set of experiments with dendrimer/DNA condensates no EGFP expression was observed, neither with VP1 capsoids nor with VP1 pentamers (data not shown). This result is in contrast to the cellular uptake experiments with the FITC-labelled DNA fragment. One possible explanation for this observation could be a decreased plasmid accessibility for RNA polymerase due to the strong dendrimer/DNA interaction. Furthermore, the electron microscopic analysis of the dendrimer/plasmid DNA loaded preparations showed an incomplete capsoid assembly which might also be responsible for an unsuccessful transfection. Finally, an EGFP expression was observed in NIH 3T3 cells treated with VP1 capsoids, which were loaded with naked plasmid DNA without dendrimers. Therefore, we conclude that the DNA condensation approach with dendrimers seems to be beneficial for the capsoid loading efficiency but has to be optimised in terms of cellular DNA accessibility.

In addition to DNA localisation and EGFP expression also the cellular VP1 protein distribution was analysed by an immunofluorescence assay (Fig. 8). In general, the uptake of VP1 was confirmed and it was shown that under different transfection conditions the distribution of the protein from the cytoplasm into the nucleus was changed. These results confirmed data obtained from the oligodeoxynucleotide uptake (Fig. 5).

Concluding our results, we demonstrated a successful intracellular uptake of DNA by an innovative carrier system, which has a high potential for further improvements of oligodeoxynucleotide as well as plasmid DNA delivery *in vitro*. With respect to possible immunogenic reactions further applications could also address the aspect of an adjuvant effect in combination with an antisense or gene therapy.

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