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Effect of cell-surface glycosaminoglycans on cationic carrier combined with low-MW PEI-mediated gene transfection

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Abstract

Glycosaminoglycans (GAGs) are negatively charged polysaccharides that are found, e.g. on cell surface. GAGs have been reported to influence gene transfection. We have previously reported that cationic lipid-mediated gene transfection can be improved by combining a small polyethylenimine (PEI) with cationic lipids. In the present study, we examined if GAGs have any effect on the synergism of small PEIs and other cationic carriers. We used wild-type CHO (GAG+) and pgsB-618 cells (GAG−). Transfection efficiency was studied using lacZ and GFP reporter genes. We found that GAGs decreased the overall level of transgene expression in a reagent-dependent manner, but the synergism caused by low-MW PEIs was less affected. There were no major differences between cell lines in cellular uptake or intracellular localization of plasmids when measured with flow cytometry and confocal microscopy, respectively. In conclusion, cell-surface GAGs interfere with transfection efficiency of different cationic reagents, but that is not necessarily related to the synergy of small PEIs and cationic lipids.

Keywords: Polyethylenimine; Liposome; Dendrimer; Gene transfection; Glycosaminoglycan

1. Introduction

Gene therapy aims to correct pathophysiological situations by delivering therapeutic genes to the body. Viral vectors are effective in gene delivery, but they may also evoke severe side effects, e.g. cytotoxicity and immunogenic responses (Wood et al., 1996; Geller et al., 1997). Therefore also, non-viral vectors are used to transfer plasmid DNA to cells. Plasmid DNA is large and negatively charged, which limits its uptake into the cells. Therefore, different carriers, such as cationic liposomes (Felgner and Ringold, 1989), polylysine and its conjugates (Wagner et al., 1991), polyethylenimines (PEIs) (Boussif et al., 1995), dendrimers (Kukowska-

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Latallo et al., 1996) and many other carriers have been used to enhance plasmid delivery.

Proteoglycans are a diverse group of molecules, which consist of a protein core and anionic glycosaminoglycan (GAG) chains (Yanagishita and Hascall, 1992). GAGs are linear, negatively charged polysaccharides. They are major components of the extracellular matrix, but are also found inside the cells and also on the cell surface in many tissues. Due to their negative charge, GAGs may interact with positively charged transfection complexes. Cell-surface GAGs were found to augment gene transfection (Mislick and Baldeschwieler, 1996; Mounkes et al., 1998) by modifying cellular uptake, but reports showing decreased transfection due to GAGs have also been reported (Ruponen et al., 2004). Extracellular GAGs can be internalized with the DNA complexes of cationic lipids and polymers (Ruponen et al., 2001). Finally, the GAGs may accumulate into the cell nuclei (Belting and Petersson, 1999a).

Recently, we demonstrated that low-molecular-weight PEIs can be combined with cationic lipids, resulting in a synergistic increase in transfection efficiency (Lampela et al., 2002, 2003). However, the exact mechanism of the synergism remains to be clarified. In this work, we took advantage of a cell line, which is not able to produce GAGs, and have studied the effect of GAGs on the gene transfection using different cationic vectors, with a special emphasis on the PEI-Dosper combination.

2. Materials and methods

2.1. Reagents

Ham's F12 medium was purchased from BioWhittaker (Belgium). Foetal bovine serum and penicillin–streptomycin were from Life Technologies (UK). Dosper and Dotap were from Roche (Germany) and SuperFect was from Qiagen (USA). Polyethylenimines, ONPG and heparan sulfate (HS) were from Sigma–Aldrich (USA).

2.2. Cell cultures

Chinese hamster ovary (CHO) wild-type cells and pgsB-618 (CHO mutant deficient in galactosyltransferase I, and thus not able to produce GAGs, ATCC CRL-2241) cells were cultured in Ham's F12 medium with 9% heat-inactivated FBS and penicillin (90 U/ml)–streptomycin (90 μg/ml).

2.3. Plasmids

Rhodamine-labeled pGeneGrip with hCMV IE promoter/enhancer driving GFP gene was purchased from Gene Therapy Systems, Inc. (USA). The BPVTKlacZ plasmid coding for β-galactosidase was synthesized by Dr. Mart Ustav (Lampela et al., 2001).

2.4. Preparation of DNA complexes

Subconfluent CHO and pgsB-618 cells were transfected as described earlier (Lampela et al., 2002, 2004). Briefly, complexes were prepared by mixing 0.2–0.5 μg DNA (1 μg DNA/30 μl 150 mM NaCl – 20 mM Hepes) with PEI solution (at an equal volume with DNA). Dosper or other cations were added (expressed later as w/w ratios) to the DNA-PEI complexes. The cation/DNA ratios were chosen based on our previous study (Lampela et al., 2004). These final complexes were pipetted to wells containing 0.4–1 ml serum-free DMEM. The transfection time was 1.5 h; thereafter, 1.0 ml (for 24-well plates) or 0.4 ml (for 8-well plates) of growth medium with serum and penicillin–streptomycin was transferred into wells and the cells were incubated for 46 h. PEI/DNA ratios are expressed as PEI amine nitrogen/DNA phosphate ratio (N/P).

2.5. β-galactosidase assay

Subconfluent cells were transfected with BPVTK-lacZ plasmid. After changing the growth medium to serum-free transfection medium, DNA complexes were pipetted into the wells as described above. After 1.5 h transfection, 1.0 ml of growth medium was pipetted into the wells, and the cells were further incubated for 46 h. Then, the cells were washed with PBS, lysed with 150 μl lysis reagent (25 mM glycylglycine, 15 mM $MgSO_4$, 4 mM EGTA, 1% Triton X-100, 1 mM DTT, 1 mM PMSF) and centrifuged at 13,000 rpm for 5 min (Eppendorf Centrifuge 5415C, Eppendorf-Netheler-Hinz, Germany). The activity of β-galactosidase was measured with an ONPG assay

from the supernatant: 4 μl of the supernatant, 96 μl of water and 100 μl of ONPG solution (2 mM $MgCl_2$, 1 mM β-mercaptoethanol, 1.33 mg/ml ONPG in 0.2 M sodium phosphate buffer) were placed in a 96-well plate and incubated for up to 1 h at room temperature. The reaction was stopped with 1 M Na_2CO_3, and samples were analysed by measuring absorbance at 405 nm with the Bio-Tek Elx-800 microplate reader (Bio-Tek Instruments, USA) and KC-4 PC-program.

2.6. Plasmid uptake and expression (rhodamine, GFP)

Rhodamine-labeled plasmid coding for GFP gene (pGeneGrip) (0.5 μg/well) was used in this study. The rhodamine label was used to visualize the amount of cells with a transgene intake, and GFP expression was used to detect the cells with a functional transgene. The transfection proceeded as described above. The cells were collected 1.5, 4.5 or 24 h post-transfection. Before collection, the cells were washed with PBS and harvested with 0.7 ml trypsin (0.25%)–EDTA (0.02%). Next, the cells were fixed with 1% paraformaldehyde (PFA) for 10 min, washed twice with 1% PFA and stored in darkness at +4 °C prior to their analysis by flow cytometry.

DNA uptake and transgene expression were analysed by flow cytometry (FACScan, Becton Dickinson) as described earlier (Ruponen et al., 2001). An argon ion laser (488 nm) was the excitation source and the fluorescence for GFP (transgene expression) was detected at 525 nm (FL1) and for rhodamine (cellular uptake) (FL3) at 670 nm. 8000–10,000 events were collected for each sample. The number of GFP- and rhodamine-positive cells was analysed from the FL1 versus FL3 dot plot by setting a gate according to a control.

2.7. Confocal microscopy

A Nikon Eclipse-TE300 (Nikon Corp, Japan) inverted microscope with laser scanning confocal equipment UltraVIEW (Perkin-Elmer, UK) was used. The cells were grown and transfected in 8-well plates (0.2 μg DNA/0.4 ml DMEM, Lab-Tek Chambered Coverglass System, Nalge Nunc, USA). After the predetermined time of incubation (1.5, 4.5 and 24 h), the cells were washed with PBS, fixed with 4% PFA, washed again twice with PBS and stored at 4 °C in the dark prior to microscopy. For the SYTO-13-stained cells, 0.5 μl of SYTO-13 (50 μM solution, Molecular Probes, USA) was added to wells 15 min before cells were washed and fixed.

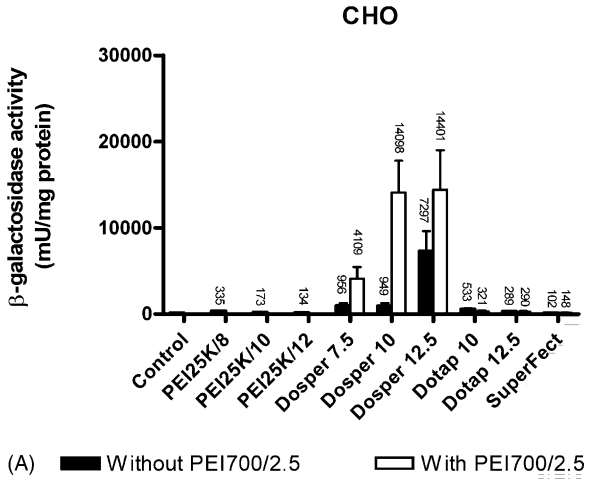
2.8. DNA condensation and relaxation

The condensation of DNA by PEI and other transfection reagents, and relaxation caused by heparan sulfate was studied by ethidium bromide (EtBr) fluorescence spectroscopy. The experiments were performed using 96-well plates and a microplate fluorescence reader (Bio-Tek FL500) at 530 nm excitation and 590 nm emission wavelengths. BPVTKlacZ in NaCl-Hepes (pH 7.4) was pipetted to 96-well plate (0.6 μg plasmid DNA/well) and ethidium bromide (0.2 μg) was added. Then, the fluorescence was read and PEI was added. After 10 min incubation, the fluorescence was read again and then, Dotap/Dosper/SuperFect were added, and after a further 10 min incubation, the fluorescence was read again. Finally, anionic HS was added to the complexes at a three-fold −/+ charge excess, and the fluorescence was read after 10 min. The PEI molecule has a number of nitrogen atoms, each having its own local environment influencing its protonability (Godbey et al., 1999a), and therefore its charge is not known. Therefore, in this work, we have used the same amount of HS in addition with PEI as added with Dosper.

3. Results

3.1. β-galactosidase activity

Without PEI700, transfection with Dosper yielded the best β-galactosidase activity in both cell lines (Fig. 1A, B). Other reagents tested (Dotap, PEI25K and SuperFect) were markedly less effective. In general, the transfection efficiency was lower in CHO cells than in pgsB-618 cells (Table 1) with all reagents studied. When PEI700 was combined with the reagents, a synergism was achieved with Dosper and SuperFect, but not with Dotap (Table 2). Furthermore, GAGs had only a minimal effect on the synergism achieved with PEI700 and Dosper (at the most effective Dosper/DNA ratio 12.5) (Table 2).



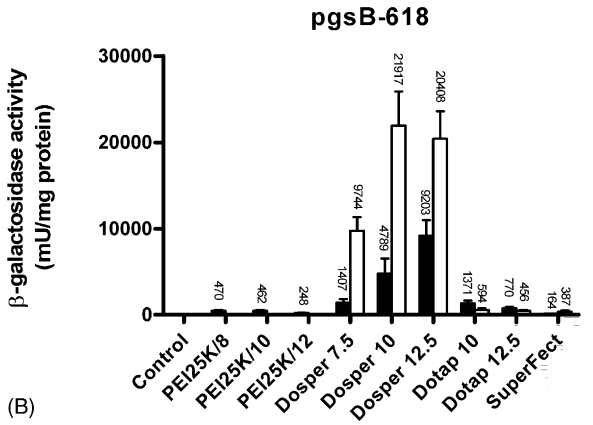


Fig. 1. The expression of β-galactosidase in the CHO and pgsB-618 cells. The cells were transfected with 0.5 μg of BPVTKlacZ plasmid and different transfection reagents as indicated. Dosper (Dosper/DNA ratios 7.5, 10 and 12.5), Dotap (Dotap/DNA ratios 10 and 12.5) and SuperFect (SuperFect/DNA ratio 30) were used without (black bars) and with (white bars) PEI700 (N/P ratio 2.5). PEI25K (N/P ratios 8, 10 and 12) was used only as such, without PEI700. After 1.5 h transfection, the growth medium was changed into wells, and the cells were further incubated for 46 h. Then, the β-galactosidase activity was measured by the ONPG assay (±S.E., $n = 4$).

3.2. Cellular uptake and GFP expression

In these experiments, a rhodamine-labeled plasmid coding for GFP was used, and it was transfected to the cells using Dosper- and Dotap-mediated transfection (with and without PEI700). In general, there were only minimal differences between CHO and pgsB-618 cell lines. However, different transfection reagents produced different results, and the most effective transfection reagent was Dosper/PEI700 combination. It produced the highest amount of rhodamine-positive cells (approximately 16%) at 1.5 h post-transfection. The amount of rhodamine-positive cells increased a bit (to 19%) at 4.5 h. All the other reagents/combinations tested resulted in 4–7% of rhodamine-positive cells.

In all cases, GFP expression was minimal at 1.5 h time point (Fig. 2A, B). However, Dosper/PEI-transfected cells started GFP production earlier than others, since GFP production was visible at 4.5 h post-transfection (Fig. 2C, D). GFP production increased with time, and was maximal at 24 h post-transfection (Fig. 2E, F). At 24 h, GFP production achieved with Dosper with and without PEI700 was superior to

Table 1
The effect of GAGs on transfection efficiency

	CHO/pgsB-618 ratio	
	Without PEI700 (%)	With PEI700 (%)
PEI25K/8	71.3	
PEI25K/10	37.5	
PEI25K/12	54.0	
Dosper 7.5	68.0	42.2
Dosper 10	19.8	64.3
Dosper 12.5	79.3	70.6
Dotap 10	38.9	54.0
Dotap 12.5	37.5	63.6
SuperFect 30	62.0	38.2

The percentage of β-galactosidase activity in CHO cells as compared to GAG-deficient pgsB-618 cells has been calculated from the results shown in Fig. 1.

Table 2
The level of synergism caused by PEI700 combined with different transfection reagents

Reagent	With PEI700/without PEI700 ratio	
	CHO	pgsB-618
Dosper 7.5	4.3	6.9
Dosper 10	14.9	4.6
Dosper 12.5	2.0	2.2
Dotap 10	0.6	0.4
Dotap 12.5	1.0	0.6
SuperFect 30	1.5	2.4

The ratio of transfection efficiency with cationic reagents (Dosper, Dotap, SuperFect (reagent/DNA ratio indicated)) with or without PEI700 has been calculated from the values presented in the Fig. 1.

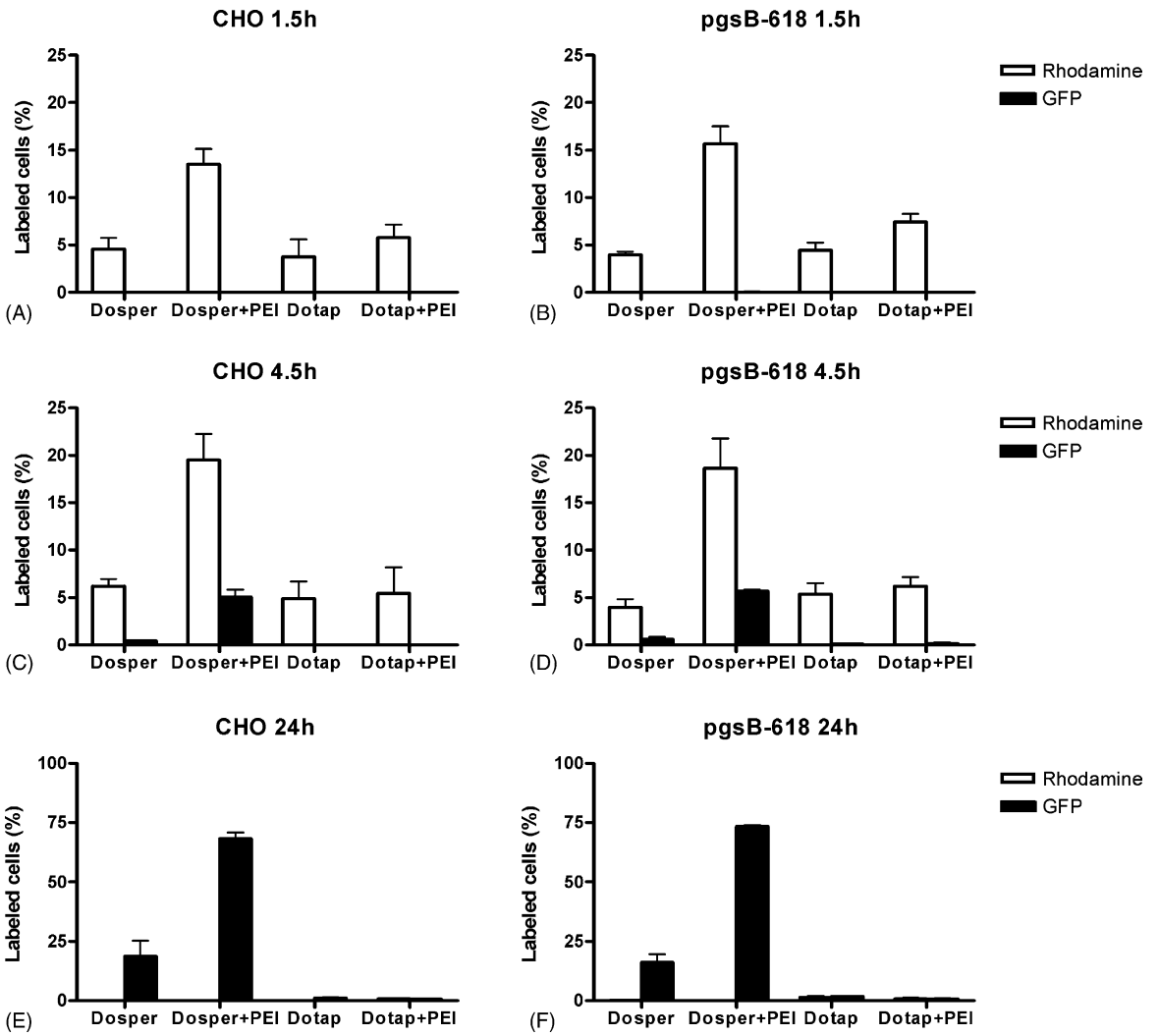


Fig. 2. Cellular uptake and expression of plasmid DNA in CHO and pgsB-618 cells. The cells were transfected with 0.5 μg of rhodamine-labeled GFP plasmid with Dosper (Dosper/DNA ratio 10), Dotap (Dotap/DNA ratio 10) or PEI 700 (N/P 2.5) combined with Dosper or Dotap. The cells were incubated in the transfection solution for 1.5 h, and then incubated for 0, 3 or 22 h in growth medium with serum. The cells were counted with FACScan (n = 3–6). Cellular uptake and expression were determined as the cells containing the rhodamine label and GFP label (±S.E.), respectively.

Dotap (GFP-positive cells 73, 19 and 2%, respectively). Dotap/PEI700 combination resulted in only 1% GFP expression.

3.3. Confocal microscopy

Confocal microscopy was used to localize the DNA-carrier complexes and expressed gene product, GFP, inside the cells. We chose two different transfection reagents, Dosper (whose efficiency was enhanced by PEI700; Fig. 1) and Dotap (efficiency decreased by PEI700) with or without PEI700 to this experiment. There were no major differences in intracellular distribution of the plasmid between the cell lines and reagents, and therefore we focus on describing Dosper-mediated transfection. Rhodamine clumps were visible

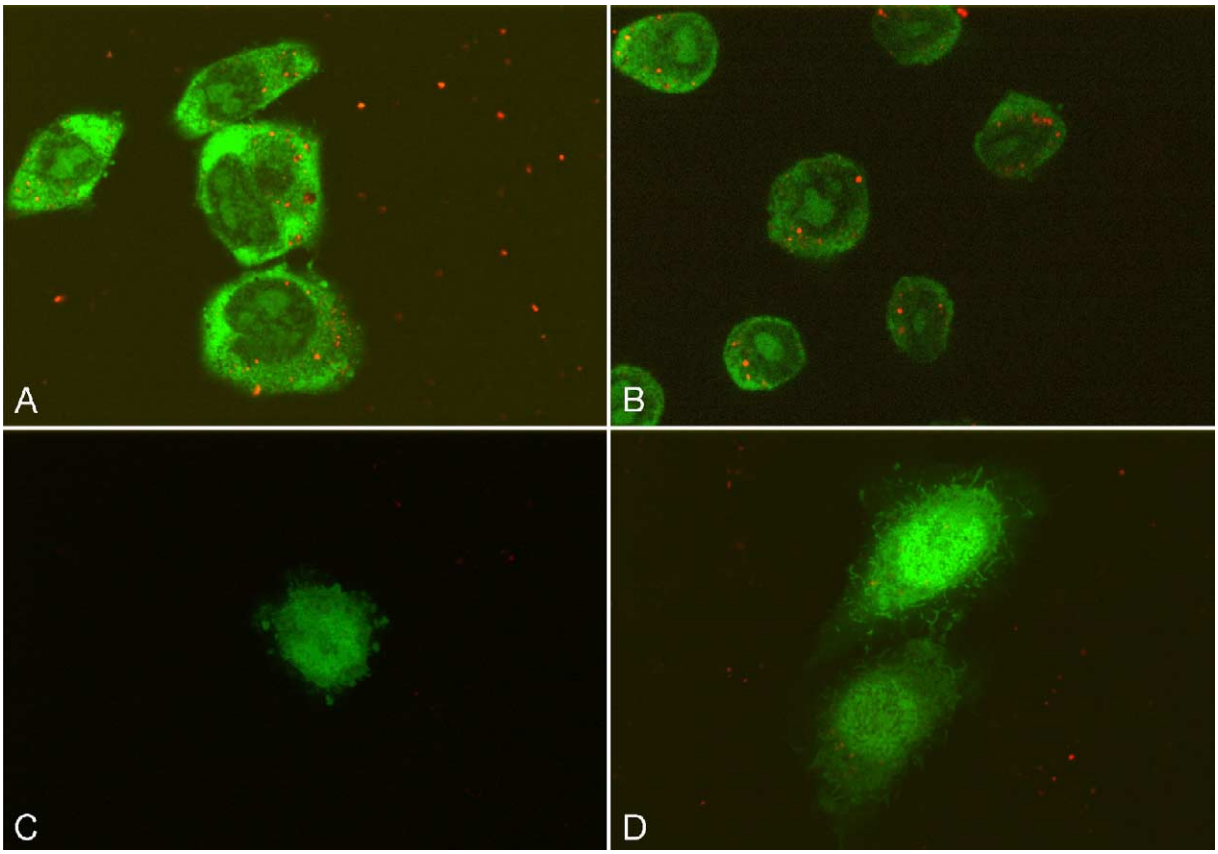


Fig. 3. The cellular uptake and intracellular trafficking of plasmid DNA in the CHO cells. The cells were transfected with Dosper as described in Section 2. Then, the cells were fixed with PFA at 1.5 (A), 4.5 (B, C) and 24 h (D) post-transfection, and studied under confocal microscopy (magnification 100×). The plasmid DNA is seen as red color. In (A) and (B), cells were stained with SYTO-13 to visualize the cell bodies prior to fixation with PFA. The green color seen in (C) and (D) is GFP produced by the transfected cells.

outside the nucleus at 1.5 h post-transfection (Fig. 3A), and a diffuse red color appeared inside the nucleus 4.5 h post-transfection (Fig. 3B). After 24 h, only a small amount of rhodamine was seen inside the cells, which is in line with the flow cytometry results. In some cases, the whole cell was covered with the red color, especially in Dosper/PEI700-transfected cells (not shown).

Significant GFP expression, i.e. filling of the whole cell, was detectable 4.5 h post-transfection (Fig. 3C) in cells transfected with Dosper with and without PEI700. The number of green cells was largest 24 h after transfection (Fig. 3D), especially in the cells transfected with Dosper/PEI700 combination. There was no difference in the localization of GFP between the two cell lines or transfection reagents.

3.4. DNA condensation and relaxation

HS was able to partly relax DNA in the complexes with PEI25K, Dosper and Dotap, but not with SuperFect (Fig. 4). Dotap itself caused the weakest condensation of DNA, the fluorescence decreased only by approximately 50% (Fig. 4A). Dosper, SuperFect and PEI25K were approximately equally effective, with fluorescence decreasing to 25% of the original value (Fig. 4B–D). Addition of HS relaxed almost all the DNA in Dosper- and PEI25K-complexes. Relaxation of DNA was a bit lower from Dotap-complexes, and HS had no effect on the SuperFect/DNA complexes. Pre-condensation of DNA with PEI700 (N/P 2.5) had no influence on the condensation or the heparan sulfate

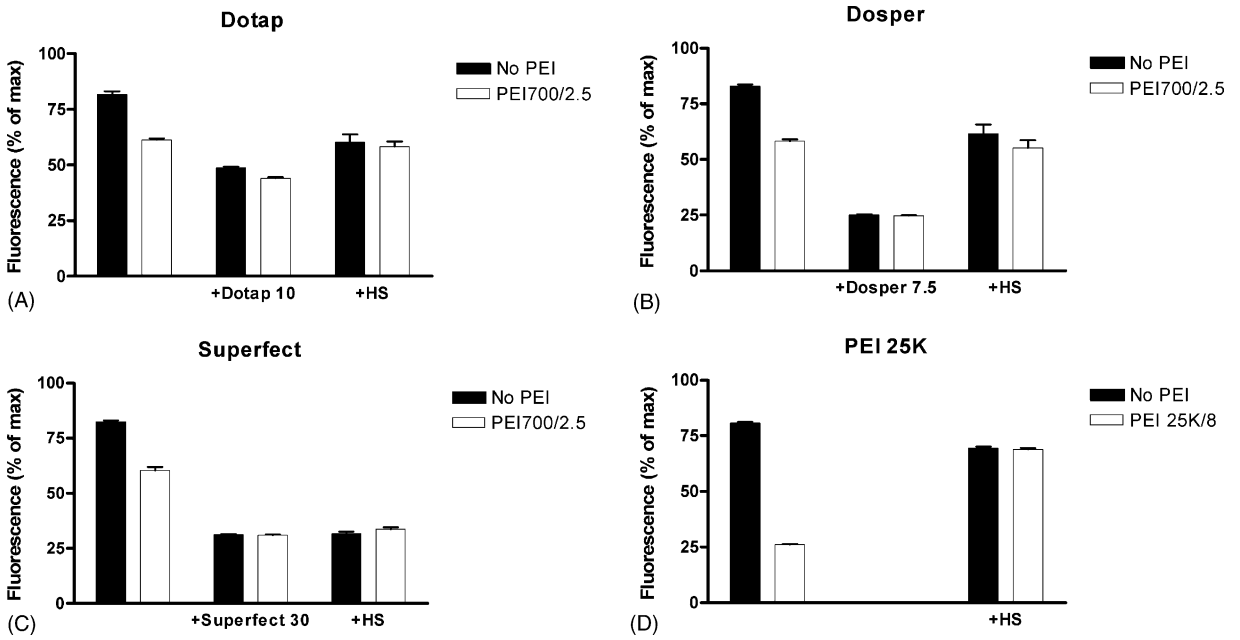


Fig. 4. The effect of HS on the DNA condensation by different transfection reagents. At first, DNA fluorescence (without any reagents) was read (equal to 100%) and PEI700 at N/P 2.5 was (white bars, A–C) or was not (black bars, A–C) added to DNA. After 10 min, fluorescence was read (first two bars), and Dotap (A), Dosper (B) or SuperFect (C) was added. After another 10 min incubation, fluorescence was read again (next two bars), after which HS was added to wells. The final fluorescence (last two bars) was read 10 min after HS addition. In Fig. 4D, DNA was condensed with PEI25K at N/P 8 (white bars). The values are percentage of the ethidium bromide fluorescence of plain DNA ± S.E. (n = 3–5).

relaxation. There were no differences in the ability of HS to relax DNA from Dosper (/PEI) complexes between Dosper/DNA ratios 7.5 and 12.5 (not shown).

4. Discussion

We have earlier demonstrated that there is a synergism between low-MW PEIs and cationic liposomes (Lampela et al., 2002, 2004), which allows high transfection efficiencies with reduced doses of the reagents. The synergism seems to depend on both the intracellular kinetics of the cells and the chemical structure of the polycation (Lampela et al., 2003, 2004). Since extracellular GAGs may affect both cellular uptake and intracellular behaviour of the complexes (Ruponen et al., 2004), we have compared a wild-type cell line and its mutant version, which lacks the ability to synthesize proteoglycans. Recently, it was shown that total content of cell-surface GAGs (including HS, chondroitin sulfate and hyaluronan) in pgsB-618 cells is less than 10% that of CHO wild-type cells (Ruponen et al., 2004). In the present study, we have focused on two questions: How do the cell-surface GAGs affect the transfection efficiency by some non-viral DNA complexes with low-MW PEIs, and is the synergism between low-MW PEIs and cationic liposomes affected by GAGs? We chose a short 1.5-h transfection time for this study, since it has been shown that cells lacking proteoglycans exhibit increased sensitivity to the cytotoxicity of cationic lipids (Belting and Petersson, 1999b).

In the present study, cell-surface GAGs inhibited transfection with all the reagents tested. These results are in agreement with the results by Belting and Petersson (1999b) and Ruponen et al. (2004). However, Mislick and Baldeschwieler (1996) and Mounkes et al. (1998) reported that GAGs enhance transfection. CHO mutant cell line pgs745 (deficient in xylosyltransferase) had 53 times lower transgene activity than in wild-type cultures (Mislick and Baldeschwieler, 1996). However, they used polylysine in their study. In general, different exogenous glycosaminoglycans,

such as hyaluronic acid and heparan sulfate, act differently on transfection (Ruponen et al., 2001). It has been shown that both hyaluronic acid and heparan sulfate inhibit PEI-mediated transfection, whereas polylysine-mediated transfection is enhanced by hyaluronic acid (Ruponen et al., 2001). Depending on a charge ratio, hyaluronic acid may also enhance Dotap- and Dotap/Dope-mediated transfection, while heparan sulfate inhibits them both. Therefore, our results are in agreement with those previously reported.

The effect of GAGs was highest when Dotap was used in transfection (Table 1). The β-galactosidase activity of Dotap-mediated transfection was approximately one-third in CHO cells when compared to GAG-deficient pgsB-618 cells. PEI25K (with the most effective N/P ratio 8), Dosper (with the most effective Dosper/DNA ratio 12.5) and SuperFect were less affected by GAGs. However, PEI25K, Dotap and SuperFect produced rather modest transfection. Dosper produced the highest β-galactosidase activity both with and without PEI700, and it was chosen for subsequent studies. Furthermore, Dosper (Dosper/DNA ratio 12.5) and the synergism produced by Dosper and PEI700 were most resistant against GAGs. This was seen in ONPG and GFP expression, as well as in cellular uptake. The uptake was further increased threefold with Dosper/PEI700-combination compared to Dosper-mediated transfection. We have reported this increase in other cell lines as well (Lampela et al., 2002, 2004). Furthermore, we have shown that PEI is necessary for the synergism, since, e.g. increase of the amount of Dosper alone does not increase the transfection (Lampela et al., 2002).

The other reagent chosen for subsequent experiments was Dotap, which was clearly affected by GAGs. The β-galactosidase activity decreased to one-third in CHO cells when compared to GAG-defective pgsB-618 cells. Furthermore, use of PEI700 with Dotap decreased β-galactosidase activity in both cell lines. These negative effects were detected also in GFP expression with flow cytometry. We have shown before that PEI has different effects on Dotap-mediated transfection depending on the cell line (Lampela et al., 2004).

The effect of cell-surface GAGs on the cellular uptake was studied with flow cytometry using the rhodamine-labeled plasmid coding for GFP. There were no differences in the uptake of the DNA complexes between the cell lines, which is in agreement with previously reported results (Belting and Petersson, 1999b). The percentage of rhodamine-positive cells indicating the presence of the plasmid was highest at 4.5 h post-transfection, and only few cells were rhodamine-positive cells at 24 h post-transfection. Probably, the DNA complexes were still in the endolysosomes, where PEI has been shown to persist (Lecocq et al., 2000). On the other hand, GFP expression increased as a function of time from 1.5 to 24 h, since protein synthesis is time-consuming. Protein synthesis seemed to start earlier with Dosper-mediated than with Dotap-mediated transfection. However, the percentage of GFP-producing cells after Dotap-mediated transfection is low even at 24 h post-transfection, so it is possible that protein synthesis is started in these cells at 4.5 h as well. However, the number of these cells is expected to be small. At both 4.5 and 24 h time points, a greater number of Dosper/PEI-transfected cells expressed GFP than Dosper-transfected cells. The increased number of GFP-producing cells transfected by Dosper/PEI complexes compared to Dosper-complexes reflects well the increase of cellular uptake seen at 1.5 h post-transfection. This is also seen in β-galactosidase assay, where PEI700 increases Dosper-mediated transfection. On the other hand, the negative effect of PEI on Dotap-mediated transfection is also visible in both β-galactosidase assay and cellular uptake/GFP production experiments.

The amount of GFP-positive cells seems to be higher than cellular uptake in Dosper-mediated transfection (with and without PEI). The exact reason for this is unknown, yet we speculate that this is due to the different detection limits for these two different probes.

The intracellular behaviour of transfection was studied with confocal microscopy. DNA was taken up by many cells, but it was mostly located in clumps outside nucleus, probably in the endosomes, as was also seen by Godbey et al. (1999b). At 4.5 h, some of the nuclei of the cells had red staining. Also, Godbey et al. (1999b) noted fluorescence inside the nuclei at 3.5 h post-transfection. However, they visualized the plasmid fluorescence in discrete, easily defined patches. PEI/Dosper synergism increases the number of stained nuclei. The highest cellular uptake and nuclear localization of DNA was seen using PEI/Dosper-complexes. PEIs are shown to direct DNA to the nucleus (Pollard et al., 1998). When Merdan et al. (2002) transfected

cells with PEI/ribozyme complexes, both PEI and ribozyme were found in the nucleus, and also transgene expression was most abundant in these cells. Merdan et al. (2002) reported that the timescale for uptake and lysosomal release of the PEI/ribozyme complexes was very heterogeneous. In the present study, the expression was clearly detected at 4.5 and 24 h post-transfection in both cell lines, respectively, and the number of colored cells was higher in Dosper(/PEI)-transfected than in Dotap(/PEI)-transfected cells. Furthermore, there were cells with a nuclei filled with diffuse red color, indicating the presence of the plasmid also without GFP expression.

HS is able to relax DNA in Dosper, Dotap and PEI complexes, but not in SuperFect complexes (Fig. 4). The almost total relaxation of Dosper/DNA complexes is due to the higher affinity of binding of spermine for the HS chains than for DNA (Belting and Petersson, 1999a). HS was also able to completely relax DNA in DNA/PEI25K complexes, but was a bit less active against Dotap/DNA complexes. This is due to the presence of the amino groups of the cations, where PEI has weaker bases (primary, secondary and tertiary) than Dotap (quaternary), and is therefore more labile upon polyanionic challenge (Ruponen et al., 1999). DNA is complexed with PEI completely at N/P 1.6, and condensed at N/P 3.0 (Petersen et al., 2002). Therefore, the N/P ratio used in this work with PEI700 (2.5) is sufficient to complex DNA, but it does not totally condense it. Therefore, pre-complexation of DNA with PEI700 prior to addition of cations did not alter DNA relaxation by GAGs, suggesting that the observed synergism is not due to decreased relaxation. This is in agreement with our previous results (Lampela et al., 2003). Furthermore, the β-galactosidase activity of Dosper (Dosper/DNA ratio 12.5) was not altered between the cell lines, although Dosper is sensitive to HS-mediated DNA relaxation. In this study, PEI700/SuperFect combination also resulted in synergism in both cell lines in contrast to SMC and C6 cell lines (Lampela et al., 2004), implying that there are also major differences between cell lines.

In conclusion, cell-surface GAGs decreased the overall transfection efficiency of different cationic lipids, low-MW PEIs and their combinations. The presence or absence of cell-surface GAGs may affect also the PEI700-mediated synergy of gene transfection, but, e.g. Dosper (ratio 12.5) was not affected. Furthermore, the influence of GAGs on the transfection by cationic carriers seems to vary with the carrier type.

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