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A new peptide vector for efficient delivery of oligonucleotides into mammalian cells

M. C. Morris, P. Vidal, L. Chaloin, F. Heitz and G. Divita*

Centre de Recherches de Biochimie Macromoleculaire, URS-155 CNRS, BP 5051, 1919 Route de Mende, 34033 Montpellier Cedex 1, France

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ABSTRACT

The development of antisense and gene therapy has focused mainly on improving methods for oligonucleotide and gene delivery into cells. In the present work, we describe a potent new strategy for oligonucleotide delivery based on the use of a short peptide vector, termed MPG (27 residues), which contains a hydrophobic domain derived from the fusion sequence of HIV gp41 and a hydrophilic domain derived from the nuclear localization sequence of SV40 T-antigen. The formation of peptide vector/oligonucleotide complexes was investigated by measuring changes in intrinsic tryptophan fluorescence of peptide and of mansyl-labelled oligonucleotides. MPG exhibits relatively high affinity for both single- and double-stranded DNA in a nanomolar range. Based on both intrinsic and extrinsic fluorescence titrations, it appears that the main binding between MPG and oligonucleotides occurs through electrostatic interactions, which involve the basic-residues of the peptide vector. Further peptide/peptide interactions also occur, leading to a higher MPG/oligonucleotide ratio (in the region of 20/1), which suggests that oligonucleotides are most likely coated with several molecules of MPG. Premixed complexes of peptide vector with single or double stranded oligonucleotides are delivered into cultured mammalian cells in less than 1 h with relatively high efficiency (90%). This new strategy of oligonucleotide delivery into cultured cells based on a peptide vector offers several advantages compared to other commonly used approaches of delivery including efficiency, stability and absence of cytotoxicity. The interaction with MPG strongly increases both the stability of the oligonucleotide to nuclease and crossing of the plasma membrane. The mechanism of cell delivery of oligonucleotides by MPG does not follow the endosomal pathway, which explains the rapid and efficient delivery of oligonucleotides in the nucleus. As such, we propose this peptide vector as a powerful tool for potential development in gene and antisense therapy.

INTRODUCTION

In the past few years major advances have been made in the fields of gene and antisense therapy with the development of a variety of oligonucleotide tools (1–4). The use of oligonucleotides as therapeutic tools in these technologies requires both that they enter target cells and that they enter at a concentration which is sufficient to reach their intracellular target and to have a significant effect (1,3,5). However, oligonucleotides are not efficiently transfered into most cells upon simple addition to the cell culture medium. The permeability of the cell membrane for nucleic acids therefore remains a major barrier to the development of gene and antisense therapies. As such, research in these fields has focused in part on the design of useful and efficient methods for the delivery of nucleic acids into cells (3,5).

At present most commonly used techniques including microinjection, transfection using cationic liposomes, viral transfection or electroporation of oligonucleotide conjugates induce stress and are each characterized by a series of limitations and drawbacks. Gene delivery mediated by cationic liposomes such as Lipofectamine^{TM}, Lipofectin^{TM}, Cytofectin^{TM} (6–9) as well as transfection mediated by polymeric DNA-binding cations such as poly-L-lysine (10,11) or polyethylenimine (12,13) are the most extensively used transfection techniques. However, they are known to present (i) sensitivity to serum, antibiotics and to certain cell culture media, (ii) cytoxicity and (iii) to be seriously limited by the overall level of transfection and the time-dependency of the experiments (3,5,8,9). Stable phosphorothioate oligonucleotides can enter the cell through the endosomal pathway but the major problem remains the permeability of the endosomal membrane. More recently, peptides derived from viral proteins, such as influenza virus hemagglutinin (14,15) or endosomal fusion protein (15), have been shown to mimic the role of these viral proteins in viral infection, in their ability to destabilize the cell membrane, and have therefore been suggested as potential agents for faciliting the delivery of nucleic acids into cells (14,15).

In the present work we describe a potent new strategy for the delivery of oligonucleotides into cells. We have designed and synthesized a peptide vector which contains both a hydrophobic domain, derived from the fusion sequence of HIV gp41, and a hydrophilic domain, derived from the nuclear localization sequence of SV40 T-antigen. This peptide vector, termed MPG, exhibits high affinity for single- and double-stranded oligo-

*To whom correspondence should be addressed. Tel: +33 4 67 61 33 92; Fax: +33 4 67 52 15 59; Email: gilles@puff.crbm.cnrs-mop.fr

The authors wish it to be known that, in their opinion, the first two authors should be regarded as joint First Authors

nucleotides and is able to deliver them into mammalian cells in <1 h, independently of the endosome pathway.

MATERIALS AND METHODS

Materials

Fluorescein and mansyl chloride were purchased from Molecular Probes. Dulbecco's modified Eagle's Medium (DMEM) and phosphate buffered saline (PBS) were from BioWhittaker. L-Glutamine, penicillin, streptomycin and trypsin were from Imperial Laboratories. Foetal calf serum (FCS) was from GIBCO BRL.

Peptide synthesis and analysis

MPG was synthesized by solid phase peptide synthesis (16) using AEDI-expensin resin with a 9050 Pepsynthetizer (Millipore UK) according to the Fmoc/tBuc method, as already described (17). MPG was purified by semi-preparative HPLC and identified by electrospray mass spectrometry and amino acid analysis (16).

Oligonucleotide synthesis and purification

Phosphodiester oligonucleotides 5′-TCCCTGTTCGGGCGCC-TC-3′ (18mer) and 5′-TGTGGAAATCTCATGC AGAGGCGC-CCGAACAGGGA-3′ (36mer), corresponding to the sequence of HIV natural primer binding site (18), were synthesized on an Applied Biosystems 380 B DNA synthesiser and purified by HPLC reverse phase chromatography (19). Double-stranded oligonucleotides (18/36mer) were formed by annealing an equimolar mixture of both single-stranded oligonucleotides in 20 mM Tris–HCl (pH 7.5) by heating for 15 min at 70°C, followed by cooling to room temperature over a period of 2 h in a water bath. Mansyl- or fluorescein-labelled oligonucleotides were synthesized and purified as already described (19,20) with a fluorescent-group attached to the last but one base at the 3′-end of the oligonucleotide 5′-TCCCTGTTCGGGCGCC*U*C-3′. The 3′-fluorescein labelled 18mer oligonucleotide was used for determination of the cellular localization of both single- and double-stranded oligonucleotides. To overcome any degradation problems due to the presence of DNases in the cell culture medium a 3′-end fluorescein-labelled-phosphorothioate oligonucleotide (5′-ACCAGCCTTCCGATCCACCAGTCATT-3′) was also used in the transfection experiments.

Fluorescence titrations

Fluorescence experiments were performed on a Spex II Jobin Yvon spectrofluorimeter. The intrinsic tryptophan fluorescence of MPG was routinely excited at 290 nm in order to minimize the substrate inner-filter effect, and the emission spectrum was recorded between 310 and 380 nm, with a spectral bandpass of 2 or 8 nm for excitation or emission, respectively. A fixed concentration of MPG (from 1×10^{-8} to 1×10^{-6} M) was titrated by increasing the concentration of each oligonucleotide (in a range of 0–200 nM) at 25°C in a buffer containing 9.4 mM PBS, pH 7.4. Extrinsic fluorescence measurements were performed by titrating a fixed concentration of fluorescently-labelled 18mer oligonucleotide (10 nM) using increasing amounts of MPG. Excitation was performed at 290 nm and the fluorescence resonance energy transfer occuring between the Trp residue and the mansyl-group was monitored at 460 nm. All measurements were corrected for the equipment and dilution as already described (19,21). Curve fitting was performed with the Grafit program (Erithacus Software Ltd) using a quadratic equation which allowed the determination of MPG/oligonucleotide ratios (19,22).

Circular dichroism

Circular dichroism experiments were performed at 25°C on a Mark V dicrograph (Jobin-Yvon, Paris) using 1 mm thick quartz cells. Spectra in the presence of lipids were recorded after 1 h incubation of MPG peptide with DOPG vesicles prepared by sonication (16).

Stability of radiolabelled oligonucleotides

Oligonucleotides were radiolabelled at the 5′ end with [γ-^{32}P]ATP using polynucleotide kinase (Boehringer Mannheim) according to the standard protocol (23) followed by Sephadex G-100 exclusion chromatography in 5 mM HEPES–KOH, 50 mM NaCl, pH 7.5. The stability of the ^{32}P-labelled oligonucleotide (1×10^{-8} M) to both nuclease and serum was measured in the absence and presence of increasing concentrations of MPG (from 1×10^{-8} to 1×10^{-5} M). Oligonucleotides were incubated for 2 and 10 h in the presence of DNase and in cell culture medium with or without serum, respectively. Oligonucleotides were then isolated by phenol extraction, ethanol precipitation and analyzed by electrophoreseis in a 12% polyacrylamide denaturing gel containing 7 M urea. The gel was dried and exposed for autoradiography.

Cell culture and transfections using MPG

Human fibroblasts HS 68 and NIH-3T3 cells were cultured in DMEM supplemented with 10% FCS as already described (24). Cells were plated on glass coverslips and grown to 75% confluency. For transfection, cells were overlaid with preformed MPG/oligonucleotide complexes (10^{-10} M/10^{-7} M) in PBS buffer, and incubated for between 30 min and 2 h at 37°C. Similar experiments were performed at low temperature (4°C) to block the cellular uptake via the endosomal pathway. The coverslips were then rinsed extensively with PBS and cells were fixed with methanol for 30 s and stained with Hoechst. The coverslips were mounted in Airvol for observations by fluorescence and confocal laser microscopy (25). The cytotoxicity of either MPG and MPG/oligonucleotide complexes was investigated in both cell lines. Cells were incubated in the presence of various concentrations of MPG or of MPG/oligonucleotide complexes in PBS buffer for 3 h, then serum was added. Cell proliferation was measured over 4 days.

RESULTS AND DISCUSSION

Design and characterization of MPG

Delivery of oligonucleotides to a subcellular target requires three major conditions to be satisfied: (i) that the cell membrane is crossed (ii) selectivity and specificity with respect to the nucleus or to the cytoplasm and (iii) high affinity and specificity for nucleic acids.

We have designed the MPG peptide in an attempt to satisfy these requirements and to produce an efficient vector for delivery of oligonucleotides into cells. MPG was designed as a 27 residue

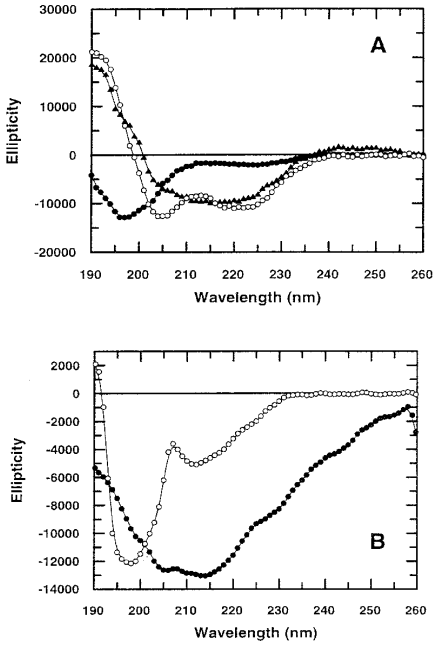

Figure 1. CD of MPG in different buffers. (**A**) CD measurements were performed in water (●), in 20% TFE (○) and in phosphate buffer with DOPG (▲). (**B**) CD experiments of MPG were performed in the presence of saturating concentrations of 18mer oligonucleotide in water (○) and in PBS (●).

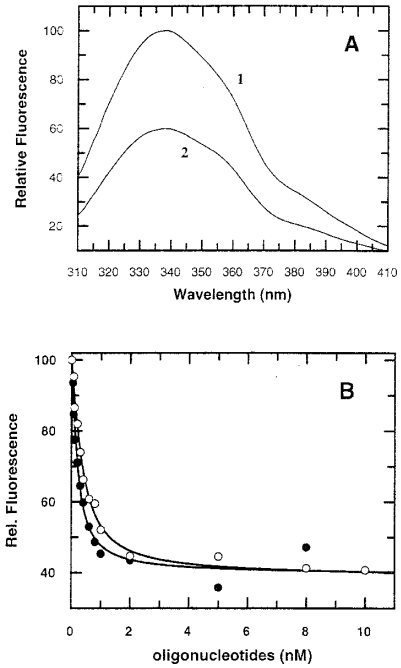

Figure 2. Binding of MPG to oligonucleotide monitored by intrinsic fluorescence quenching. A fixed concentration of MPG (1 µM) was titrated by increasing the concentration of the 18mer (●) and of the 18/36mer oligonucleotide (○). The fluorescence of MPG was excited at 295 nm and emission was monitored between 310 and 380 nm. The data were corrected and fitted as described in Materials and Methods. (**A**) Emission spectra of MPG (1 µM) in the absence of oligonucleotide (1) and in the presence of a saturating concentration of oligonucleotide (2). (**B**) Titration binding curves of MPG with single-stranded (●) and double-stranded oligonucleotide (○).

peptide 'G-A-L-F-L-G-F-L-G-A-A-G-S-T-M-G-A-W-S-Q-P-K-S-K-R-K-V' with a cysteamide group at the C-terminus and an acetyl group at the N-terminus so as to improve its ability to cross the cell membrane (17). MPG is constituted of two independent domains linked together by a short peptide sequence: (i) a hydrophobic N-terminal domain (residues 1–17: G-A-L-F-L-G-F-L-G-A-A-G-S-T-M-G-A), the sequence of which derives from the glycine-rich region of the fusion sequence of viral gp41 (26,27), known to be essential both for its membrane fusion activity and structural stabilization (26–28), and (ii) a hydrophilic C-terminal domain (residues 21–27: P-K-S-K-R-K-V) which derives from the nuclear localization signal (NLS) of the SV40 large T antigen, and which is potentially useful for improving nuclear addressing of the peptide (29,30).

MPG is chemically stable, highly soluble in physiological buffers such as PBS and exhibits a very versatile structure. As shown in Figure 1A, MPG is devoid of secondary structure when in water, but is folded into an α-helix in the presence of trifluoroethanol (20%) or into a β-sheet structure when in PBS or in DOPG vesicles. These peptide structures are not significantly modified upon interaction with single- or double-stranded DNA either in water or in PBS (Fig. 1B). It should be noted that similar results have already been described for peptides containing a hydrophobic region derived from the gp41 fusion sequence (16,28,31). The fusion peptide of gp41 has also been proposed for facilitating intracellular delivery of antisense oligonucleotides as covalent oligonucleotide–peptide conjugates (32).

Binding of nucleic acids to MPG monitored by quenching of intrinsic fluorescence

We have investigated the interaction of MPG with two different oligonucleotides: a short single-stranded oligonucleotide (18mer) and a double-stranded oligonucleotide (18/36mer) corresponding to the primer binding site of HIV (18). This region of the viral genome was of interest as it has been proposed as a target for antisense oligonucleotides (11). Both oligonucleotides were either unlabelled or fluorescently labelled with a mansyl-group attached to the last but one base of the 18mer oligonucleotide. The corresponding fluorescently labelled oligonucleotides were useful tools for determining the cellular localization of the oligonucleotides. MPG contains a single Trp residue located at position 18, between the fusion and the nuclear localization sequence motifs. This Trp residue constitutes a very sensitive probe to monitor and quantify the interaction of MPG with various nucleic acids.

The binding of oligonucleotides to MPG induced a marked quenching of the intrinsic fluorescence of MPG with a saturating value of 40%, whichever the oligonucleotide used and without modifying the maximum emission wavelength centered at 340 nm (Fig. 2A). These results suggest that upon binding of oligonucleotides to MPG, the environment of its Trp residue is not modified and that the quenching of intrinsic fluorescence observed is most likely due to direct interaction of MPG with the phosphate or with the nucleoside moiety of the oligonucleotides. As shown in Figure 2B, evidenced by the titration binding curves, MPG exhibits relatively high affinity for all oligonucleotides tested with apparent dissociation constant values in the range of 1–2 × 10^{-8} M. Similar K_d values were obtained for fluorescent- and for unlabelled-oligonucleotides, indicating that the fluorescent groups (either mansyl or fluorescein) attached to oligonucleotides

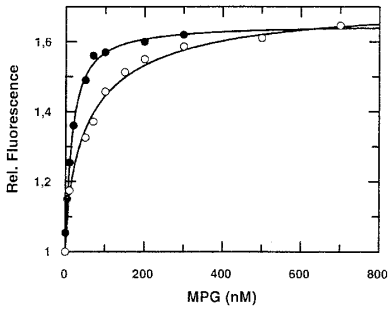

Figure 3. Binding of MPG to oligonucleotide monitored by extrinsic probe fluorescence. Fluorescently-labelled oliognucleotides were titrated by increasing the concentration of MPG. Mansyl fluorescence was monitored at 450 nm upon excitation of tryptophan residue of MPG at 290 nm. 18mer (●) and 18/36mer (○) titration were fitted using a quadratic equation as described previously.

did not interfere with the binding to MPG, and therefore validating the use of such fluorescent probes for further experiments. The affinity of MPG for single-stranded oligonucleotides was at least 2-fold higher than for double-stranded oligonucleotides (Table 1). Of interest, it should also be noted that saturation of the MPG peptide occurred at a 20–50-fold lower concentration of oligonucleotide with respect to the concentration of MPG. This can be explained either by the fact that a large part of the peptide preparation might not be folded adequately to interact with oligonucleotides, or alternatively because the ratio of MPG/oligonucleotide is effectively in the range of 20–50, depending on the nature and size of the oligonucleotides involved. That no heterogeneity of MPG was observed by intrinsic fluorescence or CD experiments and that the ratio is dependent on the size of the oligonucleotide used suggests that MPG misfolding is unlikely.

Binding of oligonucleotide to MPG monitored by extrinsic fluorescence

Binding of MPG to oligonucleotides was also monitored and quantified using a fluorescent mansyl-labelled 18mer oligonucleotide. The mansyl group, covalently linked to the base before the last at the 3′-end of the oligonucleotide is very sensitive and compatible with fluorescence resonance energy transfer with Trp fluorescence (19). As shown in Figure 3, a fixed concentration of single- or double-stranded oligonucleotides (10 nM) was titrated by increasing the concentration of MPG. Upon binding of MPG, an important transfer of fluorescence resonance energy occurred between the single Trp residue of MPG and the mansyl group attached to the oligonucleotide. In both titrations, at saturating concentrations of MPG, mansyl fluorescence increased 2-fold upon Trp excitation at 290 nm (Fig. 3). Fitting of the titration curves yielded K_d values in a nanomolar range, with a 2-fold higher value for single-stranded compared to double-stranded oligonucleotides, as already obtained using intrinsic Trp fluorescence (Table 1). Best fits were obtained for a ratio of four and eight peptide molecules per 18mer and 18/36mer oligonucleotides, respectively. Given that MPG contains four basic residues (three Lys and one Arg) in the NLS domain and taking into account electrostatic interactions, these results are in agreement with a model in which one basic residue interacts with one phosphate group of the oligonucleotide. As such, the NLS domain of MPG would be responsible for the direct interaction with oligonucleotides. In this respect, it should be reminded that the role of lysine residues in peptide/oligonucleotide interactions has been described in gene delivery using poly-L-lysine polymers (10) or lysine-rich peptides in association with a peptide derived from endosome lytic protein (14,15). Moreover, a similar 1:1 or 1:2 ratio of charge has been suggested for peptides derived from endosomal destabilizing virus protein (14).

The low values for the peptide/oligonucleotide ratio obtained by extrinsic fluorescence measurements are at least 10-fold lower compared to those estimated by intrinsic fluorescence titration. This suggests that other interactions such as peptide/peptide interactions may take place and form a larger, internalisable MPG/nucleic acid complex. In such a complex, more than one peptide would interact with an oligonucleotide and improve the formation of a peptide/peptide complex into a micro ‘particle’ of peptides surrounding the oligonucleotide. According to this model, the large fluorescence quenching of MPG could be associated either to peptide/peptide interactions or to a change in the folding of MPG induced upon binding of oligonucleotides. Since no self-association of MPG can be detected in the absence of oligonucleotide, it can be assumed that the quenching of intrinsic fluorescence is exclusively due to binding of the oligonucleotides. In fact, quenching of intrinsic fluorescence is more likely to be the sum of oligonucleotide/MPG binding and related peptide/peptide interactions.

MPG improves the stability of oligonucleotide in cell culture medium supplemented with serum

One important problem in the delivery of oligonucleotides into cells is their high sensitivity to nucleases and low stability in cell culture medium supplemented with serum. The impact of the interaction of MPG with oligonucleotide was investigated by following the degradation of oligonucleotide by nuclease in the presence or not of MPG. Radiolabelled oligonucleotides were incubated for 2 h in the presence of DNAse and for 10 h in cell culture medium with or without serum. Oligonucleotides were then isolated by phenol extraction, ethanol precipitation and analyzed on denaturing polyacrylamide gel electrophoresis. As shown in Figure 4, the 18mer radiolabelled oligonucleotide is stable in the absence of serum (lane 2) and rapidly degraded by either DNase (lane 3) or serum (lane 4). In contrast, when oligonucleotide is preincubated with MPG before adding DNase (lane 5), no significant degradation is observed, suggesting that MPG strongly protects the oligonucleotide. MPG also protected the oligonucleotide from the nuclease in the cell culture medium supplemented with serum (lane 6). That oligonucleotides retain their integrity when complexed to MPG is in perfect agreement with the high affinity of MPG for the oligonucleotide and the formation of a micro ‘particle’ of peptide surrounding the oligonucleotide.

Table 1. Affinity parameters of MPG for single- and double-stranded oligonucleotides

Oligonucleotide	Intrinsic fluorescence		Extrinsic fluorescence	
	K_1 (nM)	ΔF (%)	K_2 (nM)	MPG/oligo
18mer	5.0 ± 0.5	38	–	–
18/36mer	12 ± 0.8	40	–	–
18mer[a]	4.3 ± 0.9	41	12 ± 1.8	3 ± 1
18/36mer[a]	8.6 ± 1.2	40	23 ± 2	8 ± 2

The affinity for the fluorescently-labelled or non-fluorescently-labelled oligonucleotides was determined by quenching of the intrinsic fluorescence of MPG or by an increase in extrinsic fluorescence of the mansyl-group attached to the 18mer oligonucleotide, as described in Materials and Methods. Titration curves were fitted using quadratic equations. K_1 and K_2 correspond to the apparent dissociation constants monitored by intrinsic and extrinsic fluorescence, respectively.
[a]Fluorescently labelled oligonucleotides.

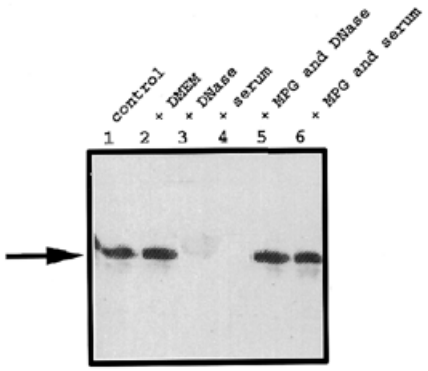

Figure 4. Stability of oligonucleotides in the presence of MPG. Control ^{32}P-radiolabelled oligonucleotide is shown in lane 1. ^{32}P-Radiolabelled oligonucleotides were incubated for 2 h in the presence of DNase (lane 4) and for 10 h in cell culture medium with (lane 3) or without (lane 2) serum. Oligonucleotides were then isolated by phenol extraction, ethanol precipitation and analyzed on denaturing polyacrylamide gel electrophoresis. Similar experiments were performed with oligonucleotide preincubated with MPG before adding DNase (lane 5) or cell culture medium containing serum (lane 6).

MPG promotes large-scale delivery of oligonucleotides into mammalian cells

We have evaluated the ability of MPG to deliver both single- and double-stranded oligonucleotides into the fibroblastic HS68 and NIH-3T3 cell lines. Phosphorothioate oligonucleotides were also used to reduce the risk of degradation of the oligonucleotide in the cell culture medium. Fluorescein-labelled oligonucleotides were complexed with MPG (0.1 μM) in a ratio of 1:20 (oligo/MPG), corresponding to a ratio of one phosphate group for five basic residues of MPG, according to the results obtained by fluorescence titrations, and overlaid on coverslips of cultured HS68 and NIH-3T3 fibroblasts for between 30 min and 1 h. Experiments were first performed at 37°C and the extent of oligonucleotide delivery as well as their cellular localization was monitored by fluorescence microscopy and confocal lazer microscopy (Fig. 5). As shown in Figure 5C and E, in the presence of MPG fluorescently labelled 18mer phosphodiester and 26mer phosphorothioate oligonucleotides were rapidly driven into cells after 30 min incubation. Complete delivery is achieved after 1 h incubation in >90% of cells. Essentially the same results were obtained for double-stranded phosphodiester oligonucleotides (Fig. 5G). In all cases, the localization of the oligonucleotide was mainly nuclear (Fig. 5E and G). In the absence of MPG, oligonucleotides were taken up by the cell at very low levels; 1 h

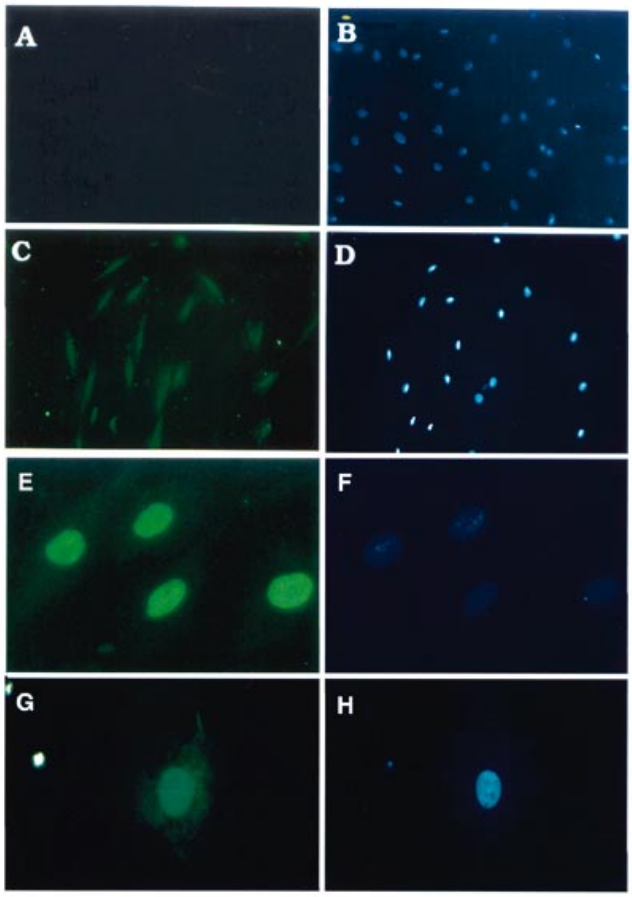

Figure 5. MPG-mediated delivery of oligonucleotides in human fibroblasts. Fluorescein-labelled single- or double-stranded oligonucleotides were complexed with MPG (0.1 μM) in a ratio of 1:20 (oligo/MPG) and cellular localization was monitored by fluorescence microscopy. (**A**) and (**B**) Control experiments with fluorescently-labelled phosphorothioate oligonucleotide in the absence of MPG. (**C**), (**D**), (**E**) and (**F**) MPG-mediated cellular delivery of single-stranded oligonucleotide. (**G**) and (**H**) MPG-mediated cellular delivery of double-stranded oligonucleotide. (B), (D), (E) and (H) correspond to nuclear staining using Hoechst 33258.

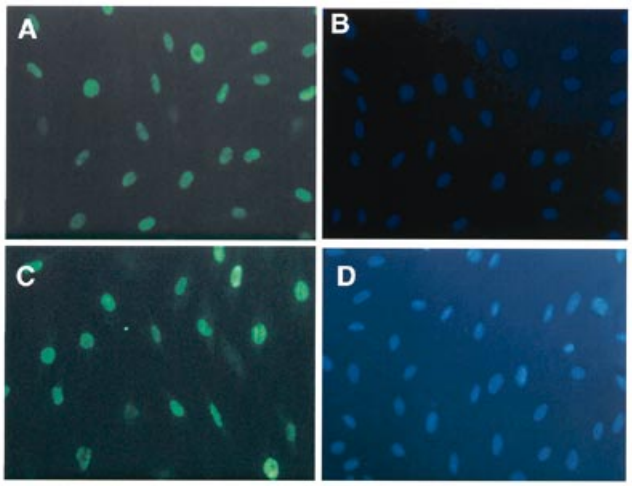

Figure 6. MPG-mediated delivery of oligonucleotides in human fibroblasts at low temperature. Fluorescein-labelled single oligonucleotides were complexed with MPG as described in Figure 5 and cellular localization was monitored by fluorescence microscopy. (**A**) and (**B**) The control experiments were performed at 37°C. (**C**) and (**D**) MPG-mediated cellular delivery of single-stranded oligonucleotide at 4°C.

after incubation, most of the internalized phosphorothioate oligonucleotides were located in endosomal compartments, the lack of nuclear labelling confirmed that free oligonucleotides were unable to cross the endosomal membranes.

Cellular and nuclear delivery of oligonucleotide by MPG in <1 h suggests that internalisation is faster that endocytosis events. In order to understand the mechanism of cell delivery, similar transfection experiments with MPG were performed with HS68 fibroblasts at low temperature (4°C) to block the endosomal pathway. As shown in Figure 6, at 4°C oligonucleotides were also rapidly internalised into cells (in <1 h), and localized to the nucleus. This result suggests that the cellular uptake of oligonucleotide mediated by MPG does not require endosome-derived vesicles and shows that MPG increases the permeability of cell membranes for the oligonucleotide. Together with the fact that MPG enhances the stability of oligonucleotides against nucleases, this result explains the ability of MPG to deliver oligonucleotides into the nucleus on a large scale. We compared the advantages of MPG-mediated delivery of oligonucleotides into cells with that of the more commonly used Lipofectamine^{TM}-mediated transfection technique (7,8). Several parameters favour MPG, including the efficiency of transfection, the lack of sensitivity to serum and to cell culture medium, and the speed of transfection. Finally, in both the NIH 3T3 and the HS68 cell lines no cytotoxicity of either MPG or of MPG/oligonucleotide complexes were detected up to a concentration of 100 μM. Phosphorothioate and phosphodiester oligonucleotides alone were not cytotoxic up to 1×10^5 M.

CONCLUSIONS

In the present work we have described a new strategy for oligonucleotide delivery into mammalian fibroblast cells. This strategy is based on the use of a peptide vector, MPG, which exhibits relatively high affinity for single- and double-stranded oligonucleotides.

The MPG peptide vector was designed by combining the fusion sequence of HIV gp41 (26,28) and the nuclear localization sequence of SV40 T antigen (29,30). When MPG is mixed with oligonucleotides in solution, they rapidly associate into a complex with tight non-covalent interactions. By simply overlaying this MPG–oligonucleotide complex onto mammalian cells, efficient delivery of the complex into the nucleus of the cells occurs in <1 h. From our results, we have suggested that rapid self-assembly between MPG and oligonucleotides involves mainly electrostatic interactions, which take place between the negative charges of the nucleic acids (mainly phosphate groups) and the positively charged moiety of MPG, mainly the lysine residues of the NLS. Such a mechanism, involving electrostatic interactions has been previously suggested in poly-L-lysine- or lysine-rich peptide-mediated oligonucleotide transfection techniques (10,14). Based on these and on our results we propose a model of interaction between MPG and oligonucleotides in which primary, main binding would occur through electrostatic interactions involving the basic residues of the NLS, whilst the ability to cross the cell membrane would be conferred by the hydrophobic gp41-derived domain of MPG. Moreover, our data indicate a high MPG/oligonucleotide ratio (>20), which suggests that oligonucleotides are most likely coated with several molecules of MPG. Only one part of the MPG molecule would be in direct contact with an oligonucleotide molecule, whilst the other would interact directly with another MPG molecule, itself also bound to the oligonucleotide. Formation of such a supra-molecular, capsid-like complex increases the stability of oligonucleotides by protecting them from extracellular and cellular nucleases.

MPG constitutes a powerful tool for oligonucleotide delivery into cells. It is stable in physiological buffers and promotes the delivery of oligonucleotides into cells in <1 hour with >90% efficiency. The MPG-based strategy improves both the stability and the cellular uptake of oligonucleotides independently of the endosomal pathway. This strategy overcomes the limitations and hazards which arise in the commonly-used techniques for oligonucleotide delivery into mammalian cells, including transfection mediated by cationic lipids (6–8) and by viral vectors (3). Moreover, MPG exhibits no cytotoxicity and bypasses sensitivity to serum, a feature which reduces the length of time-dependency encountered in other transfection techniques considerably.

Work is currently in progress to optimize stability and efficiency of MPG in order to produce a potent tool for research which could have an impact both on antisense technology and in gene therapy.

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