# Required Structure of Cationic Peptide for Oligonucleotide-binding and -delivering into Cells

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Abstract: Improvement of the methods for oligonucleotide delivery into cells is necessary for the development of antisense therapy. In the present work, a new strategy for oligonucleotide delivery into cells was tested using cationic peptides as a vector. At first, to understand what structure of the peptide is required for binding with an oligonucleotide, several kinds of $\alpha$-helical and non-$\alpha$-helical peptides containing cationic amino acids were employed. As a result, the amphiphilic $\alpha$-helix peptides were best for binding with the oligonucleotide, and the long chain length and large hydrophobic region in the amphiphilic structure of the peptide were necessary for the binding and forming of aggregates with the oligonucleotide. In the case of non-$\alpha$-helical peptides, no significant binding ability was observed even if their chain lengths and number of cationic amino acid residues were equal to those of the $\alpha$-helical peptides. The remarkable ability of oligonucleotide delivery into COS-7 cells was observed in the $\alpha$-helical peptides with a long chain length and large hydrophobic region in the amphiphilic structure, but was not observed in the non-$\alpha$-helical peptides. It is considered that such $\alpha$-helical peptides could form optimum aggregates with the ODN for uptake into cells. Based on these results, the $\alpha$-helical peptide with a long chain length and large hydrophobic region is applicable as a vector for the delivery of oligonucleotides into cells. Copyright © 2000 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: cationic peptide; $\alpha$-helical peptide; oligonucleotide; cell; delivery

## INTRODUCTION

The use of antisense oligonucleotides (ODNs) is a novel approach to down regulate specific gene products [1,2]. The antisense ODNs have been expected as therapeutic tools for inhibiting viral replication due to their specificity for viral RNA sequences. Nevertheless, the poor permeability of the cell membrane for the ODNs remains a major barrier to the

development of the antisense therapy. Therefore, research in these fields has been focused in part on the design of useful and efficient methods for the delivery of ODNs into cells [2,3]. To date, many molecules such as cationic liposomes [4,5], lipo-polyamines [6], poly(L-lysine) [7,8], polyamidoamine dendrimers [9] and DEAE-dextran [10] have been successfully used to deliver and increase the cellular uptake of ODNs into cells.

Recently, Morris *et al.* reported a new strategy for ODN-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 the SV40 T-antigen [11]. In addition, Wyman *et al.* also reported a peptide vector, termed KALA (30

residues), which is an amphiphilic peptide derived from the sequence of the amino-terminal segment of the HA-2 subunit of the influenza virus hemagglutinin involved in the fusion of the viral envelope with the endosomal membrane [12]. Peptides are now easily available due to the development of an automatic peptide synthesis apparatus and reagents. Therefore, it is expected that the functional gene carrier molecules such as carbohydrate-modified peptide for targeted ODN-delivery can be freely design and synthesized.

The authors have studied gene transfection systems based on amphiphilic $\alpha$-helix peptides consisting of 18–24 amino acids, including six to seven arginines (e.g. Ac-LARL-LARL-LARL-LRAL-LRAL-LRAL-NHCH$_3$) [13,14]. From the results of these studies, it was found that these peptides could bind to a plasmid DNA, and form large aggregates with the DNA competent to be internalized into cells via the endocytosis pathway. In addition, the hydrophobic region of the peptides was found to play an important role in the formation of the aggregates and disruption of the endosomal membrane for the escape from degradation in endocytic vesicles.

In this study, the authors tested whether these amphiphilic $\alpha$-helical peptides are applicable for the delivery of ODNs, of which molecular weights are extremely smaller than the plasmid DNA, into cells. Furthermore, to understand what structure of a peptide is required for ODN binding and delivery into cells, these abilities were investigated for several kinds of $\alpha$-helical and non-$\alpha$-helical peptides containing cationic amino acids.

## EXPERIMENTAL PROCEDURES

### Materials

Reagents used for the synthesis and analysis were of reagent grade. Amino acid derivatives were purchased from Watanabe Chemical (Hiroshima, Japan). Alamer Blue™ was purchased from Wako chemicals (Osaka, Japan). COS-7 cells (a monkey kidney cell line, RCB accession no. RCB0539) were purchased from RIKEN Cell Bank (Tsukuba, Japan). Dulbecco's modified Eagle's medium (DMEM), media supplements, and heat inactivated fetal calf serum (FCS) were from IWAKI GLASS (Chiba, Japan).

### Peptide Synthesis

The peptides were synthesized by the 9-fluorenyl-methoxycarbonyl (Fmoc) procedure on a ABI 431A peptide-synthesizer (Applied Biosystems, Foster, CA). The synthesized peptides were purified by HPLC (Hitachi L7100 system, Tokyo, Japan) with reversed-phase column (YMC-Pack ODS or YMC-Pack C4, $10 \times 250$ mm). Elution was carried out with a linear gradient established between 50 and 100% acetonitrile in 0.05% trifluoroacetic acid for 30 min monitored at 220 nm. The final products were identified by fast atom bombardment mass spectrometry (FAB-MS) using a JEOL JMS-DX303 mass spectrometer (Tokyo, Japan) and matrix assisted laser desorption ionization mass spectrometry (MALDI TOF-MS) using a Shimadzu Kratos Kompact MALDI III apparatus (Kyoto, Japan).

### Oligonucleotide

A chemically synthesized 12-mer oligodeoxyribonucleotide (5′-AGGTTTAGGATT-3′), that was double-labeled with rhodamine X at its 5′-end and fluorescein at its 3′-end, was used (R-ODN-F), and the 12-mer oligodeoxyribonucleotide (5′-AGGTTTAG-GATT-3′), that was labeled with fluorescein at its 5′-end (F-ODN), was also used. These oligonucleotides were supplied by Bex (Tokyo, Japan). The sequence of the oligonucleotide corresponds to that of an effective antisense oligonucleotide against hepatitis C virus (HCV) gene expression [15].

### Nuclease Protection Assay

The tests were performed as described by Uchiyama *et al.* [16]. The peptides and R-ODN-F (3.0 µg) were mixed in 30 µl of HBS (21 mM Hepes–NaOH buffer (pH 7.4) containing 135 mM NaCl, 5.0 mM KCl) at a charge ratio of 6.0 (positive (peptide)/negative (R-ODN-F)). After 30 min at 30°C, 5 units of Bal 31 nuclease (Takara Shuzo, Shiga, Japan) were added. After 40 min at 30°C, the reaction was stopped by addition of 20 µl of 0.1 M EDTA to the reaction mixture. Before measurement of fluorescence intensity, 950 µl of 20 mM Tris–HCl buffer (pH 7.4) containing 0.1% SDS was added to the sample. The fluorescense spectra were recorded at an excitation wavelength of 494 nm and an emission wavelength of 515 nm (fluorescein) and 605 nm (rhodamine) with a fluorescense spectrophotometer (Hitachi F-3010, Tokyo, Japan). The percentage of the inhibition by addition of the peptides was evaluated by the equation: $100 - (100 \times (R - R_0)/(R_{100} - R_0))$, where $R$ is the ratio of fluorescent intensity at 517 and 605 nm, $R_0$ and $R_{100}$ are the ratio of intensities in the absence of Bal 31 and the peptide, respectively.

## Circular Dichroism

Circular dichroism (CD) spectra were recorded on a JASCO J-720 W spectropolarimeter using a quartz cell of 1.0 mm path length. The peptides were dissolved at the concentration of 20 μM in HBS. Measurements were performed in the presence of the oligonucleotide (ODN, 5′-AGGTTTAG-GATT-3′) at a peptide/ODN chage ratio of 4.0 and in the absence of the ODN.

## Electron Microscopy

Samples were prepared by mixing the peptide and ODN (5′-AGGTTTAGGATT-3′) in 500 μl of HBS. These were then left standing for 30 min at room temperature. Peptide–ODN complexes were processed for transmission electron microscopy (TEM) using a negative stain technique. Sixty-microliter drops of freshly prepared samples were placed on glow-discharged carbon-coated 200-mesh copper grids for 3 min. The solution was wicked off with filter paper and replaced with 1% aqueous uranyl acetate for 30 s. After removal of the solution, grids were rinsed in distilled water and allowed to dry. Grids were imaged in a JEOL JEM100S TEM.

## Transfer of Oligonucleotide into Culture Cells

COS-7 cell, a simian virus kidney cell line transformed with simian virus 40 (SV40), were grown to just before confluence in 16-mm dishes in DMEM with 10% FCS and 100 μg/ml streptomycin in an atmosphere of 5% $CO_2$ at 37°C. Peptide (64.8 μM as cationic charge concentration) and the 12-mer F-ODN (2.7 μM) were mixed in 250 μl of HBS (peptide/F-ODN charge ratio = 2.0) and allowed to stand for 30 min at 37°C. The mixture was poured gently onto the cells. After incubation for 3 h at 37°C, 1 ml of DMEM with 10% FCS was added. The cells were incubated for 3 h from the first addition of the medium and were washed in PBS, fixed for 30 min in 3% paraformaldehyde at room temperature, and analysed using a OLYM-PUS BX60 fluorescence microscope (Tokyo, Japan).

## RESULTS AND DISCUSSION

## Peptide Structures

To understand what structure of a peptide is required for binding with an oligodeoxyribonucleo-tide, several kinds of $\alpha$-helical and non-$\alpha$-helical peptides containing cationic amino acids were employed (Table 1). As $\alpha$-helical peptides, two series of peptides were examined. One is a series of peptides basically consisting of repeating units of 'LARL' abbreviated as $4_3$ and $4_6$ series peptides [13,17]. Peptides $4_3$S and $4_6$S are designed on the basis of the $4_3$ and $4_6$ sequences so as to reduce the hydrophobicity of $4_3$ and $4_6$ by introducing three and six Ser residues, respectively, instead of the Leu and Ala residues. Peptide $4_6$P contains the Pro[8]-Pro[9] sequence, which can disrupt the $\alpha$-helical structure instead for Leu[8]-Leu[9] of $4_6$ in the middle of the peptide chain. The $4_3$ and $4_6$ series peptides have three and six residues of cationic amino acids (Arg), respectively, in the molecules. The other is a series of peptides consisting of 18 residues abbreviated as Hel series peptides [14,18]. The Hel series peptides are composed of Leu and Lys residues in the ratios of 5:13, 7:11, 9:9, 11:7, and 13:5 (Hels 5-13, 7-11, 9-9, 11-7, and 13-5, respectively). The amphiphilic $\alpha$-helical structures of these peptides have a systematically varied hydrophobic–hydrophilic balance.

As non-$\alpha$-helical peptides, two series of peptides were also examined. One is a series of peptides consisting of repeating units of 'SPRR', which is observed in the amino- and carboxyl-termini of histone H1 and the amino-terminus of histone H2B from sea urchin sperm, abbreviated as HM (histone motif) series peptides [19]. HM-2, -4 and -6 have two, four and six repeating units of 'SPRR', respectively. The other is a series of peptides consisting of repeating units of 'RPPF', which is observed in bactenecin 5 isolated from the granules of bovine neutrophils [20]. Bactenecin 5 is known to have a poly(proline)-II structure in aqueous solution [21]. A series of repeating peptides was prepared, Ac-(RPPF)$_n$-NHCH$_3$ ($n = 2$, 6, 10), abbreviated as BM (bactenecin 5 motif) series peptides [22].

All synthetic peptides could be identified by mass spectrometry (Table 1).

## Binding Ability of Peptide with DNA

To examine the binding abilities of the peptides with ODN, the inhibition abilities of the peptides were analysed against degradation of the R-ODN-F (rhodamine-5′-AGGTTTAGGATT-3′-fluorescein) by Bal 31 nuclease. The degradation of the R-ODN-F by the nuclease could be monitored by fluorescent resonance energy transfer between the rhodamine

Table 1  Structures of Cationic Peptides and Identification Using Mass Spectrometry

| | | Molecular weight [M+H]⁺ | |
| | | Calculated | Found |
| --- | --- | --- | --- |
| α-Helical peptides | | | |
| $4_3$ | Ac-LARL-LARL-LARL-NH$_2$ | 1419 | 1419[a] |
| $4_3$S | Ac-LARS-LARS-LSRL-NH$_2$ | 1383 | 1383[a] |
| $4_6$ | Ac-LARL-LARL-LARL-LRAL-LRAL-LRAL-NH$_2$ | 2781.4 | 2781.7[b] |
| $4_6$S | Ac-LARS-LARS-LSRL-LRSL-SRAL-SRAL-NH$_2$ | 2709.1 | 2708.3[b] |
| $4_6$P | Ac-LARL-LARL-LARP-PRAL-LRAL-LRAL-NH$_2$ | 2749.3 | 2749.1[b] |
| Hel 5-13 | KKLK-KLKK-KWKK-LKKK-LK | 2324.0 | 2323.5[b] |
| Hel 7-11 | KKLK-KLLK-KWKK-LLKK-LK | 2294.0 | 2293.2[b] |
| Hel 9-9 | KLLK-KLLK-LWKK-LLKK-LK | 2264.0 | 2263.4[b] |
| Hel 11-7 | KLLK-LLLK-LWKK-LLKL-LK | 2233.9 | 2234.9[b] |
| Hel 13-5 | KLLK-LLLK-LWLK-LLKL-LL | 2203.9 | 2204.2[b] |
| Non-α-helical peptides | | | |
| HM-2 | Ac-SPRR-SPRR-NH$_2$ | 1051 | 1051[a] |
| HM-4 | Ac-SPRR-SPRR-SPRR-SPRR-NH$_2$ | 2044 | 2044[a] |
| HM-6 | Ac-SPRR-SPRR-SPRR-SPRR-SPRR-SPRR-NH$_2$ | 3039.5 | 3040.2[b] |
| BM-2 | Ac-RPPF-RPPF-NHCH$_3$ | 1068 | 1068[a] |
| BM-6 | Ac-RPPF-RPPF-RPPF-RPPF-RPPF-RPPF-NHCH$_3$ | 3059.7 | 3060.2[b] |
| BM-10 | Ac-RPPF-RPPF-RPPF-RPPF-RPPF-RPPF-RPPF-RPPF-RPPF-RPPF-NHCH$_3$ | 5050.1 | 5050.5[b] |

[a] Molecular weight determined by fast atom bombardment mass spectrometry (FAB-MS).
[b] Molecular weight determined by matrix-assisted time of flight mass spectrometry (MALDI TOF-MS).

and fluorescein as described by Uchiyama [16]. Figure 1 shows the relative inhibition activities of the peptides. For the $4_3$ and $4_6$ series peptides, all peptides except $4_3$S showed high inhibition activities of more than 70%. Among the Hel series peptides, only Hel 13-5, which has the widest hydrophobic region, showed a 70% inhibition activity while the other Hel series peptides did not show any significant inhibition. These results indicated that the chain length of at least 12 residues and/or a wide hydrophilic region in the amphiphilic structure of the α-helical peptide was required for strong binding with the ODN.

In the case of non-α-helical peptides, no inhibition abilities were observed in the HM series peptides. However, the inhibition ability of the BM series peptides was increased by increasing the chain length of the peptide, which then, reached the 50% inhibition level in the case of BM10. This result indicated that for binding of the non-α-helical peptide with the ODN, a chain length longer than that of the α-helical peptide was necessary, and the binding depended on the peptide sequence, that is, the 'RPPF' sequence is favorable for binding with the ODN rather than the 'SPRR' sequence.
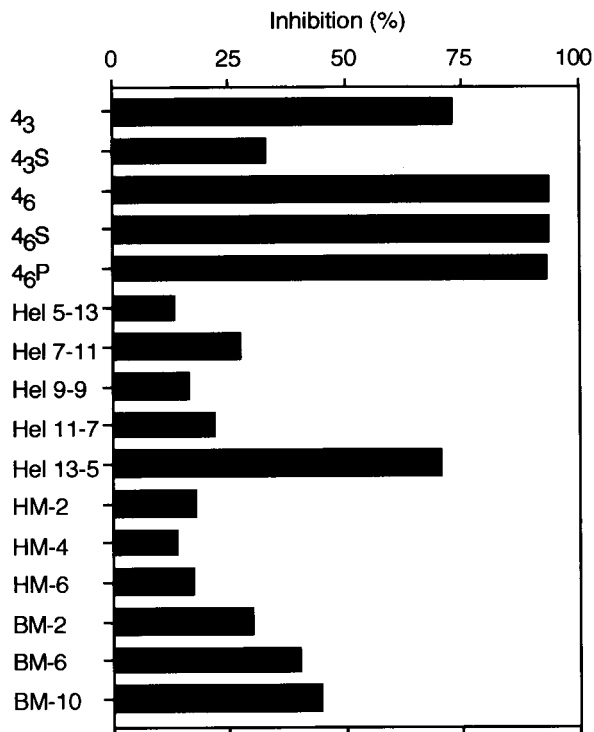


Figure 1  Inhibition activities of the peptides against digestion of the double-labeled fluorescent oligonucleotide (R-ODN-F) by Bal 31 nuclease.

## Structural Change in Peptide

To analyse the conformational change in the peptide that occurred during the binding with the ODN, the CD spectra of the peptides were measured in the absence and presence of an ODN (5′-AGGTT-TAGGATT-3′) at a charge ratio of 4 (Figure 2). In these measurements, it was hard to analyse spectra under 205 nm due to a large absorbance. As shown in Figure 2(A), in the absence of the ODN, the $\alpha$-helical peptides with a long chain length, $4_6$, $4_6S$ and $4_6P$, showed the typical $\alpha$-helix CD pattern with double minima at 208 and 222 nm in the HBS at a peptide concentration of 20 μM, while the peptides with a short chain length, $4_3$ and $4_3S$, mainly took a random structure. In the case of the Hel series peptides, the peptides with a wide hydrophobic region in the amphiphilic structure, Hels 11-7 and 13-5, had a highly $\alpha$-helical structure, while the peptides with a wide hydrophilic region, Hels 5-13, 7-11 and 9-9, mainly had a random structure (Figure 2(B)).

In the presence of the ODN, remarkable changes in the CD spectra of $4_6$, $4_6S$, $4_6P$, Hels 11-7 and 13-5 were observed (Figure 2(C and D)). The common change in the spectra of these peptides with the addition of the ODN was that the valley at 208 nm became shallower. In these figures, in order to avoid confusing the data containing both the spectra of the peptide and the ODN, we showed the CD spectra of the peptides at a peptide/ODN charge ratio of 4.0, where the CD spectrum originated in the ODN was significantly smaller than that in the peptides. On the other hand, in the case of $4_3$, $4_3S$, Hels 5-13, 7-11, and 9-9, no remarkable changes in the CD spectra were observed. The spectra changes observed in the peptides with a long chain length and/or wide hydrophobic region in the amphiphilic structure indicates that the conformational changes in the peptides occurred with the addition of the ODN. Furthermore, the spectral changes also suggested formation of aggregates between the peptides and the ODN because the changes were similar to that in the case of aggregates between the peptides and a plasmid DNA as previously reported by the authors [13,14].

Furthermore, the conformations of the non-$\alpha$-helical peptides in the absence and presence of the ODN were also analysed. All HM series peptides showed random structures in the absence and presence of the ODN, therefore, no spectral changes were observed (data not shown). All BM series peptides showed a typical CD pattern of the poly(proline)-II structure with a valley at 208 and a shoulder at 222 nm as reported by Niidome *et al.* [22] in the absence of the ODN. In the presence of the ODN, significant spectral differences were not observed from that in the absence of the ODN (data not shown). The BM series peptides with a long chain length showed nuclease inhibition abilities suggesting that these peptides could interact with the ODN without conformational changes.

## Aggregate Formation of Peptide with Oligonucleotide

To assess the structure of the peptide–ODN complex, transmission electron microscopy with negative staining was used. Figure 3 shows the complexes of the peptides and ODN (5′-AGGTTTAG-GATT-3′) at a charge ratio of 2.0. In the case of the $4_6$ series peptides, $4_6$ formed twisted fiber-like aggregates with the ODN (Figure 3(A)). Peptides $4_6S$ and $4_6P$ also formed aggregates similar to $4_6$. Although $4_3$ generated aggregates similar to the $4_6$
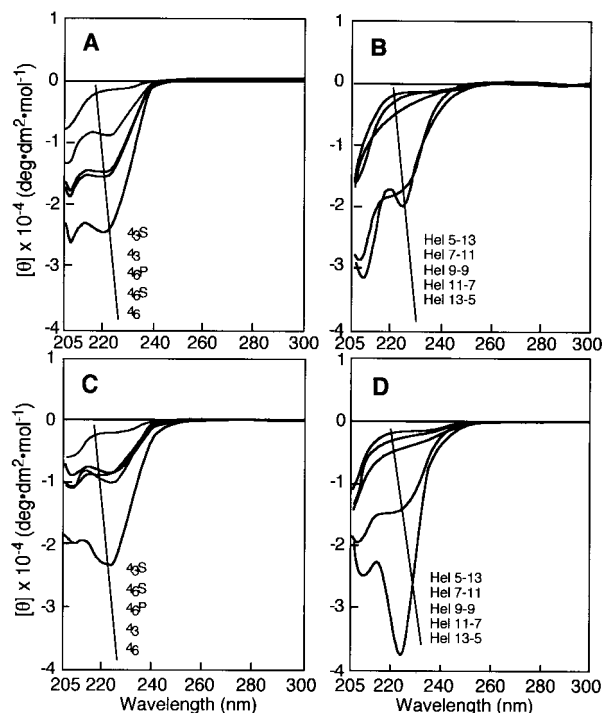


Figure 2  Circular dichroism spectra of the peptides. The peptide was dissolved at 20 μM in HBS. Measurement was performed in the absence (panels A and B) or in the presence of the ODN (panels C and D) at 25°C. Panels A and C show the spectra of the $4_3$ and $4_6$ series peptides. Panels B and D show the spectra of the Hel series peptides.
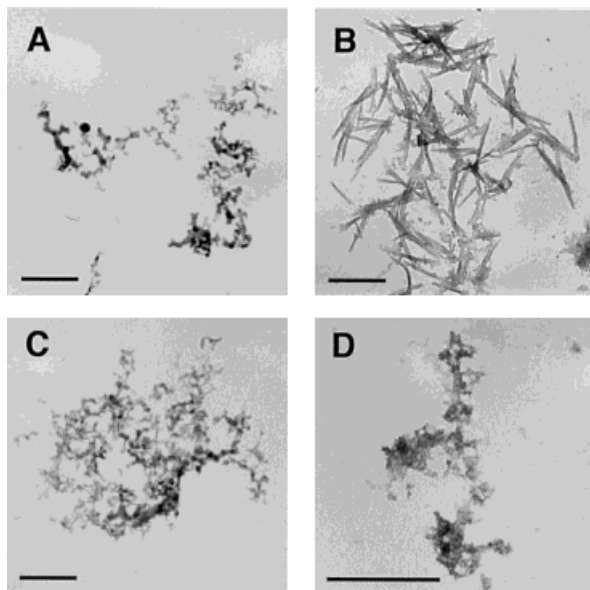
Figure 3 Electron photomicrographs of the peptide–ODN complex. Panels A–D show examples of aggregates formed by $4_6$, Hel 13-5, Hel 11-7 and BM-10, respectively. Bar indicates 1.0 μm.

series peptides, the population of the aggregates was much lower (data not shown). In the case of $4_3$S, no aggregates were observed. This result indicated that an amphiphilic $\alpha$-helix peptide with a long chain length is advantageous for the formation of aggregates with the ODN. In the case of the Hel series peptides, Hel 13-5 interestingly formed needle-like aggregates with the ODN (Figure 3(B)), whereas aggregates of Hel 11-7 were similar to those of $4_6$ (Figure 3(C)). Although this difference in the shape of the aggregates could not be fully explained, it is reasonable to suppose that the stronger intermolecular interaction of Hel 13-5, which has a larger hydrophobic region compared with Hel 11-7, occurred, and as a result, such characteristic aggregates of Hel 13-5 were formed. On the other hand, peptides with large hydrophilic regions, i.e. Hels 5-13, 7-11 and 9-9, did not show clear aggregates. This result indicates that not only the number of cationic charges but also the width of the hydrophobic region in the peptide plays an important role in the formation of aggregates with the ODN.

BM-10 also formed twisted fiber-like aggregates similar to those of $4_6$ with the ODN (Figure 3(D)), whereas no aggregates were observed in all the HM series peptides and BM-2. Although BM-6 generated aggregates similar to BM-10, the density of the

image and the population of the aggregates were lower (data not shown). This result indicates that even for a non-$\alpha$-helical peptide, extension of the chain length allows aggregate formation with ODN. Since the BM series peptides have several phenylalanine residues with an aromatic side chain, it is likely that a $\pi-\pi$ interaction occurred between the peptide and the ODN in addition to an electrostatic interaction. This multiple interaction would increase the ability of aggregate formation with the ODN.

## Cell Uptake of Peptide–Oligonucleotide Complex

The uptake by mammalian cells of the peptide–ODN complex was examined using the COS-7 cells. Cells were preincubated in DMEM containing 10% FCS on sterilized cover slips. The serum-free medium was replaced, and then, the free F-ODN (fluorescein-5′-AGGTTTAGGATT-3′) or the complex of the peptide and the F-ODN was added to a final concentration of 2.7 μM (F-ODN) in HBS at a charge ratio of 2.0. As shown in Figure 4, the cellular uptake of the complexes was remarkably observed in the case of $4_6$. It is considered that the F-ODN was localized in mainly the endocytic vesicles and slightly in the nucleus because a strong dot-like fluorescence in the cytosol and a weak round fluorescent image correspond to the location of the nucleus observed in the cells (Figure 4(A and B)). On the other hand, about 5% of the cells, in which the F-ODN was localized mainly in the nucleus, was observed (Figure 4(C and D)). The cellular uptake of a phosphorothioate oligonucleotide (F-S-ODN; fluorescein-5′-AGGTTTAGGATT-3′) was also examined, which is resistant to degradation by nucleases. As a result, images similar to the case of the F-ODN were observed (data not shown). The remarkable uptake of the F-ODN was also found in the case of Hel 13-5, however, other peptides including all non-$\alpha$-helical peptides did not show any abilities to deliver the F-ODN into cells. As a matter of course, no uptake was observed in F-ODN only. These results suggest that the amphiphilic $\alpha$-helix structure and the large hydrophobic region in a peptide are necessary for the uptake of the ODN-complex into the cells.

For this experiment on the ODN transfer into cells, the similar fluorescence images were observed in the cases of peptide/DNA charge ratios of 2.0, 4.0 and 6.0. However, at the charge ratios of 4.0 and 6.0, significant cytotoxicity of the peptides was observed as will be described in the following section,
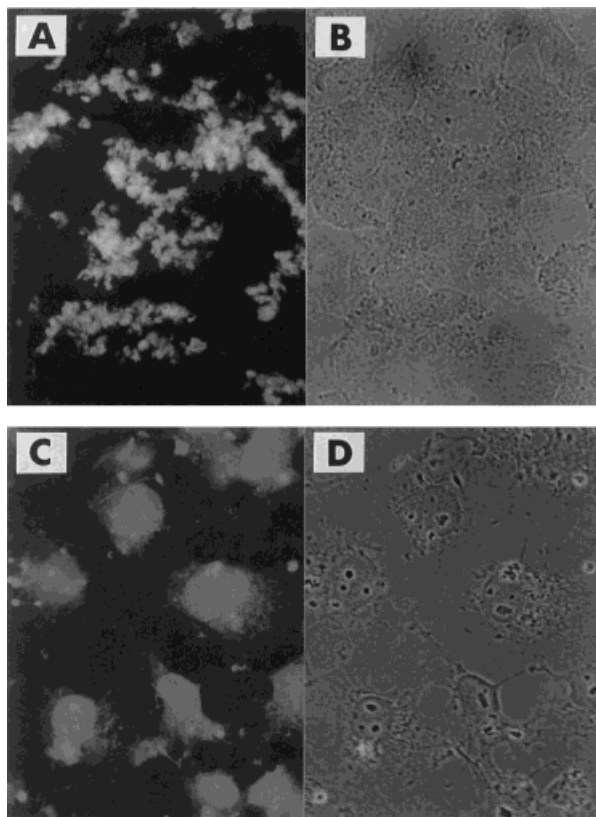
Figure 4 Cellular uptake of F-ODN mediated by $4_6$. The paired images of the fluorescent component (photos A and C) and transmitted-light images (photos B and D) were photographs taken from the same cells.

and accordingly, the conditions using a mixture at a charge ratio of 2.0 were selected. On the other hand, in the nuclease protection assay, differences in the character of peptides could not be cleared in the case of mixing at a charge ratio of 2.0. As we would like to focus the differences of the character of the peptides, the data at a charge ratio of 6.0 were employed in the nuclease protection assay.

Previously, we examined the binding abilities of these peptides to plasmid DNA and their gene transfection efficiencies into COS-7 cells [13,14]. As a result, the binding ability was observed in $4_3$, $4_6$, $4_6S$, $4_6P$ and all Hel series peptides, and then the transfection ability was observed in $4_6$, $4_6S$, $4_6P$ and Hels 9-9, 11-7 and 13-5. Compared with the results obtained from the present study on the ODN binding and the cellular uptake, the binding ability of the peptide with the ODN was weaker than that with the plasmid DNA. Furthermore, the peptides with ODN transfer abilities into cells were limited compared with the peptides with that of the plasmid

DNA. This result indicates that a stronger binding ability of the peptide with nucleic acids is required for stable binding of the peptide with the ODN, which is a smaller molecule compared with the plasmid DNA.

## Cytotoxicity of Peptide or Peptide–Oligonucleotide Complex

Cytotoxic activities of the complexes of peptides and ODN (5′-AGGTTTAGGATT-3′) were evaluated using Alamer Blue™ under the same conditions as those in the transfection procedure at charge ratio of 2.0. In the case of only the peptide, $4_3$, $4_6$, $4_6S$, $4_6P$, Hel 11-7 and Hel 13-5 showed appropriate cytotoxicities (40–60% cell viability), while significant cytotoxicities were not observed in the other peptides including the non-$\alpha$-helical peptides (Figure 5, cross-hatched bar). However, in the case of the complex with the ODN, the cytotoxicities of the peptides, which showed cytotoxicities in the absence of the ODN, decreased (about 75% of cell viability and up) (Figure 5, closed bar). It is
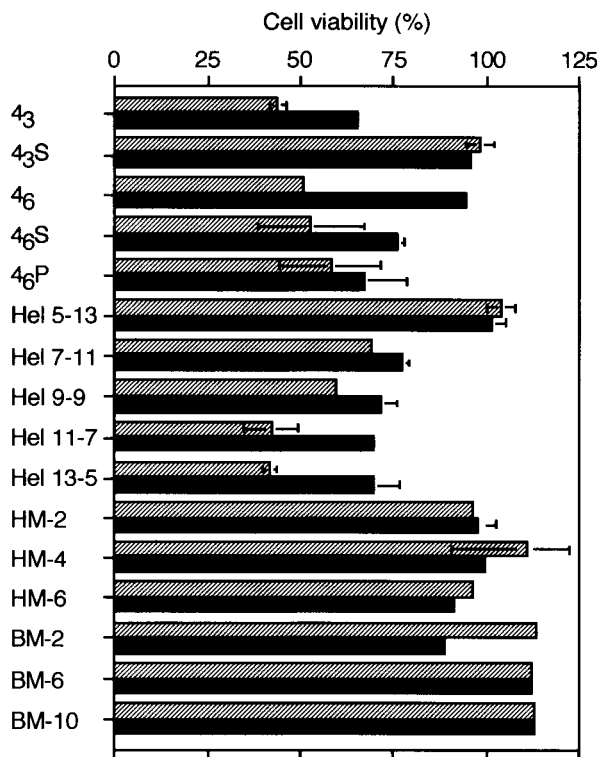


Figure 5 Cytotoxic activities of the peptides–DNA complexes evaluated using Alamer Blue™ under the same conditions as those in the transfection procedure. Cross-hatched bars and closed bars indicate the cell viabilities in the presence of the peptide alone and the peptide–ODN complex, respectively.

considered that decreasing the perturbating activity of the peptides against the cell membrane by forming the complex with the ODN reduced the cytotoxicities of the peptides [17,18]. Practically, in the cases of peptide/DNA charge ratios of 4.0 and 6.0, strong cytotoxicity of the $\alpha$-helical peptides was observed due to excess amount of the peptide relative to the ODN.

## CONCLUSIONS

In this study, it was tested whether a cationic peptide is useful as a vector for the efficient delivery of ODN into cells. First, the peptide structure required for binding with the ODN was examined. As a result, it is clear that the amphiphilic $\alpha$-helix peptide was the most advantageous for binding with the ODN, and its binding ability depended on the chain length and the width of the hydrophobic region in the amphiphilic structure of the peptide. In addition, such an $\alpha$-helical peptide could form large aggregates with the ODN. In the case of non-$\alpha$-helical peptides, significant binding ability was not observed even if their chain lengths and number of cationic amino acid residues were equal to that of the $\alpha$-helical peptides. The ODN-transfer ability into COS-7 cells was observed in the $\alpha$-helical peptides with a long chain length and wide hydrophobic region. Although further study is necessary to clarify the detailed transfer mechanism, it is considered that such peptides could form optimum aggregates with the ODN for uptake into cells due to their intermolecular hydrophobic interaction. From this point of view, such an $\alpha$-helical peptide could be said to be advantageous as a simple vector for efficient delivery of ODN into cells compared with the non-$\alpha$-helical peptide.

## Acknowledgements

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