Development of A Novel Cell-penetrating Peptide for Intracellular Delivery of Proteins and Liposomes


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ABSTRACT SUMMARY

A ganglioside GM3-binding peptide GBPc01 selected from phage-displayed peptide libraries was employed as cell recognition device for the delivery of proteins and liposomes. An avidin-biotinylated GBPc01 complex, a GBPc01 fusion green fluorescent protein (GFP), and a stearoyl-GBPc01-modified liposome were prepared, and the interactions with Hela cells (or B16 cells) were investigated. They were efficiently internalized into cells by caveolae-mediated endocytosis. It was indicated that the GBPc01 peptide was useful as a novel cell-penetrating peptide.

INTRODUCTION

Cell-penetrating peptides such as TAT and R8 have been intensively examined for the transfer of proteins, genes and liposomes through cell membrane [1]. In our previous study, pentadecapeptides having high affinity for ganglioside GM3 (Neu5Ac-Gal-Glc-Cer) were identified from phage-displayed random peptide libraries. One of the GM3-binding peptides, GBPc01 (GWWYKGRAPVSAVA), bound to sialylgalactose moiety on the cell membrane, and inhibited influenza virus infection [2]. In the present study, an avidin-biotinylated GBPc01 complex (Av-bGBPc01), a GBPc01 fusion green fluorescent protein (GBPc01-GFP), and a stearoyl (C18)-GBPc01-modified liposome (GBPc01-Lipo) were prepared, and the interaction of GBPc01-containing proteins and liposomes with Hela cells were investigated, and the function of GBPc01 as a cell-penetrating peptide was evaluated.

EXPERIMENTAL METHODS

Ganglioside GM3-binding peptides were selected from phage-displayed peptide libraries using a GM3-containing monolayer (Figure 1). Biotinylated peptides and C18-peptides were synthesized on an automated peptide synthesizer using standard Fmoc chemistry. For the modification of biotinyl and stearoyl groups, Fmoc-Lys(biotin)-OH (Novabiochem) and stearic acid were linked to the C-terminal and N-terminal, respectively. The purity and structure of the modified peptides were verified by HPLC and MALDI-TOF/MS.

Avidin-biotinylated peptide complexes were prepared by mixing biotinylated-GBPc01 (or biotinylated TAT) with FITC-labelled avidin at molar ratio of 4:1. GFPs fused with GBPc01 or TAT peptides were prepared using plasmid vectors coding peptide-GFP. The peptides and His-tag were conjugated with the C-terminal of GFP. Peptide fusion GFPs were purified by Ni-affinity chromatography, and the purity was verified by SDS-PAGE.

Liposomes composed of egg PC, cholesterol and fluorescein-DHPE (F-DHPE) were prepared by thin film-hydration method. Then, the liposome suspension was mixed with C18-
RESULTS AND DISCUSSION
Quantitative analysis by FCM indicated that Av-bGBPc01 complexes were internalized into Hela cells immediately after binding to cell surface (Figure 3). On the other hand, the amount of avidine-biotinylated TAT (Av-TAT) complexes internalized into cells for 90 min was only 30% of complexes adhered on the cell surface. Since the interaction of Av-bGBPc01 complexes with Hela cells was inhibited by the addition of sialic acid (Figure 4), it was suggested that the Av-bGBPc01 complexes specifically bound to sialic acid of cell surface. Furthermore, cellular uptake of Av-bGBPc01 complexes was largely inhibited by the addition of methyl β-cyclodextrin. Furthermore, observation by CLMS indicated that Av-bGBPc01 complexes were co-localized with anti-caveolin1 antibodies. Therefore, it was indicated that the Av-bGBPc01 complexes were dominantly taken up through raft/caveolae-dependent endocytosis. On the other hand, Av-TAT complexes were internalized into cells through the both of raft/caveolae-endocytosis and macropinocytosis.

Cell uptake efficiencies of GFPs fused with GBPc01 or TAT peptides were also investigated using HeLa cells. GBPc01-GFPs were taken up more efficiently than TAT-GFPs. And, GBPc01-GFPs were specifically bound to sialic acid of cell surface, and were taken up through raft/caveolae-dependent endocytosis. Furthermore, interactions of C18-peptide-modified liposomes with B16 cells were investigated, and sequence-specific cellular uptake of GBPc01-Lipo was observed by CLMS and FCM.

CONCLUSION
A GM3-binding peptide GBPc01 selected by phage-display method was employed to deliver proteins and liposomes into cells. The GMBe01-mediated cellular uptake was sugar specific, and dominant cellular uptake pathway was raft/caveolae-dependent endocytosis. Therefore, it is indicated that the GBPc01 peptide is a novel cell-penetrating peptide.

REFERENCES

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