Phosphatidylserine-selective Conformational Change of α-Helix Peptide, Td3717, and Its Ability to Transfect Cancer Cells

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A synthetic peptide, Td3717, showed selective affinity for anionic phospholipids, phosphatidylserine and phosphatidylglycerol, and formed an amphiphilic α-helical structure. This specificity originated not only from electrostatic interactions, but also from the primary amino acid sequence and overall structure of the peptide.

To date, many chemically synthesized cationic compounds have been used as gene carrier molecules.[1,2] Peptides are promising candidates as functional gene carriers due to their participation in a wide variety of biological activities. By taking advantage of these diverse activities, effective peptide-derived functional gene carriers could be constructed. Indeed, cationic α-helix peptides derived from viral fusion peptides have been reported to act as gene transfection agents.[3–5] A cell-penetrating peptide, the TAT peptide, originating from a viral RNA binding protein, was employed to enhance the internalization of DNA.[6,7] The RGD peptide was used as a targeting ligand for αvβ3-integrin over-expression in the neovasculature of tumors.

Previously, we reported bifunctional synthetic peptides Td3701 and Td3717, which acted as polycations that bound DNA, and as ligands for phosphatidylserine (PS) for targeted delivery to tumor cells expressing PS on the cell surface.[8–11] These peptides were based on the amino acid sequence of the carboxy-terminal C2 domain of the human coagulation factor VIII (FVIII), which is known to contribute to anchoring of FVIII on the surface of PS-exposed activated platelets.[12] Td3717 was constructed by optimizing the sequence of Td3701 to enhance the transfection efficiency without losing specificity.[11]

Td3717 is an amphiphilic α-helical peptide containing cationic amino acids.[11] The cationic and hydrophilic face of the α-helix structure contributes to electrostatic binding with DNA and the hydrophobic face stabilizes the complex and interacts with the lipid membrane of the cell. After uptake into the cells, the peptide also aids in the escape of the DNA from the endosome into the cytosol. Furthermore, the overall structure of the peptide is likely to contribute to the specific recognition of PS on the cell surface. In this study, to understand the specific transfection ability of Td3717 to PS-exposed cells, we investigated the interaction of Td3717 with several anionic phospholipids, including PS.

To examine specific binding of Td3717 to PS, we employed two control peptides, KALA and Hel 11-7,[3,5] that form amphiphilic structures like Td3717, bind to phospholipid membranes, and show transfection abilities (Figure 1).

Td3717:
TRYLRLHPRSWVHQLALRLRYLRLHPRSWVHQLALRS

KALA:
WEAKLAKALAKALAKHLAKALAKALKACEA

Hel11-7:
KLLKLLLKLWKKLLKLLK

Figure 1. Amino acid sequences of cationic α-helix peptides.

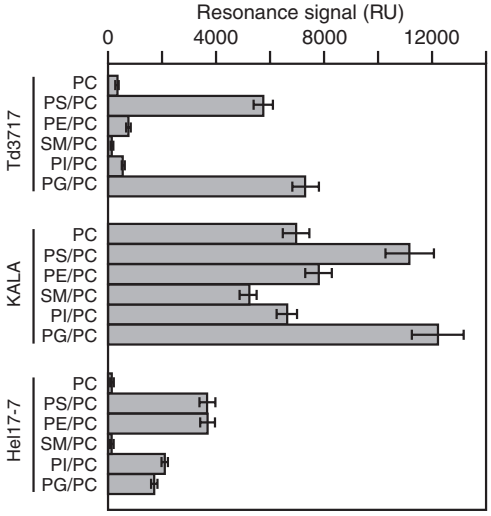

Figure 2. Binding abilities of cationic peptides with phospholipid membranes. Peptide solutions were introduced into a sensor chip containing the membranes. After washing with 0.01% BSA/PBS, the remaining resonance signals were evaluated. Data represent mean values for $n = 3$ and bars are standard deviations of the means.

The binding abilities of the peptides with PS were evaluated by surface plasmon resonance (SPR) using the BIAcore 3000 system. Phospholipid membranes comprising PC (phosphatidylcholine; neutral lipid) alone and mixtures of 30% of PS (anionic lipid), PE (phosphatidylethanolamine; neutral lipid), SM (sphingomyelin; neutral lipid), PI (phosphatidylinositol; anionic lipid), or PG (phosphatidylglycerol; anionic lipid) to 70% with PC (abbreviated to PS/PC, PE/PC, SM/PC, PI/PC, and PG/PC, respectively in Figure 2) were prepared and immobilized on the sensor chip (L1) using 0.2 mM phospholipid solution, followed by blocking solution (0.01% bovine serum albumin (BSA)/

PBS). Peptide solutions (10 μM) in 0.01% BSA/PBS were introduced into the housing of the sensor chip. The chips were washed with 0.01% BSA/PBS and the remaining resonance signals were evaluated. As shown in Figure 2, Td3717 showed affinity for some of the anionic phospholipid membranes, especially PS/PC and PG/PC. In contrast, Td3717-scr, which has a scrambled sequence of Td3717; TLRY-RPSH-QLRL-RAVL-HLWL-RYRP-SHQL-RLRA-VLHW-S and has a random secondary structure, showed no affinity to any phospholipid membranes (data not shown),[11] indicating that the affinities of Td3717 for PS/PC and PG/PC originated not only from electrostatic interactions but also from the overall structure of the peptide. The control peptide, KALA, showed strong signals in the presence of all the phospholipid membranes, demonstrating that KALA has no selectivity for any of the phospholipids tested. Hel 11-7 showed affinity with all anionic phospholipids (PS/PC, PI/PC, and PG/PC) and PE/PC. PE, which has a neutral and small hydrophilic head, alters the packing of lipid bilayers. As a result, the accessibility of Hel 11-7 would increase to the same level as the anionic membranes that interact with the peptide electrostatically.

The structural features of Td3717 were evaluated by circular dichroism (CD) spectrum analysis, in the presence or absence of 1 mM liposomes comprising PC alone, PS/PC, PE/PC, SM/PC, PI/PC, and PG/PC (Figure 3). In the presence of PS/PC, PG/PC, and PI/PC liposomes, Td3717 showed a large valley at around 222 nm, indicating that Td3717 adopted a partial α-helical structure. In the presence of PC alone, PE/PC and SM/PC liposomes, low levels of α-helix formation were observed, and no α-helical structure was observed in the absence of liposomes. In contrast, Td3717-scr showed no α-helical structures in the presence or absence of liposomes (data not shown). In the CD measurements, we observed an interaction between Td3717 and PI/PC, which was not observed in the SPR experiment shown in Figure 2. In the SPR measurements, 0.01% BSA was added to the samples. It is reasonable to suppose that BSA bound nonspecifically to PI and disturbed the binding of Td3717 to PI. In the case of PS, BSA might not have affected the binding of the peptide to PS due to the peptide's stronger affinity for PS than PI.

In summary, we demonstrated that Td3717 has selective affinity for PS and PG, originating not only from electrostatic interactions but also from the primary amino acid sequence and overall structure of the peptide. The specificity did not originate simply from the secondary structure of the peptide, such as an amphiphilic α-helix.

In a transfection experiment using Td3717,[11] a DNA complex with Td3717 interacted with the phospholipid membrane. Although binding of DNA might interfere with the recognition of PS on the cell surface, the specificity of the complex could be improved using a slight excess of the peptide when mixed with the DNA at C/A ratio (cations of peptide and anions of the DNA) of 2.5. In contrast, we cannot ignore the possibility that some peptide structure already present on the DNA might be advantageous in the specificity for PS.

PG is a rare component of the phospholipid bilayer of the plasma membrane of the mammalian cell, therefore we might conclude that the selective recognition by Td3717 of PS-exposed on the cell surface is triggered by the structural change of Td3717 to an α-helix. This mechanism is unique, and is quite different to that of well-known cationic amphiphilic α-helix peptides.

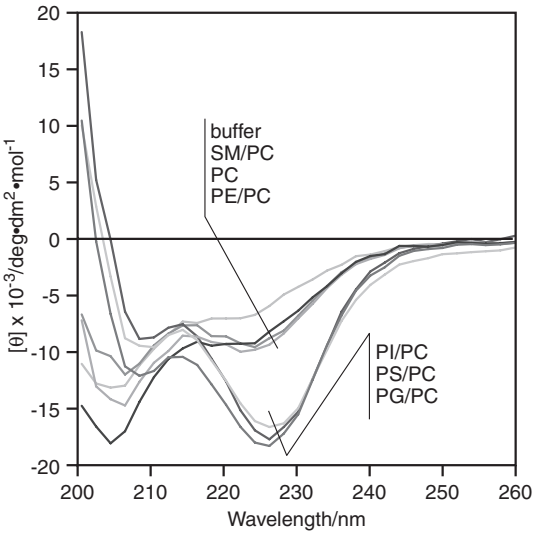

Figure 3. Structural changes of Td3717 in the presence of phospholipids. CD spectra of Td3717 at a concentration of 20 μM were measured in the presence or absence of 1 mM liposomes comprising PC alone, PC/PS, PE/PC, SM/PC, PI/PC, and PG/PC.

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