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Interaction of Amphiphilic α-Helical Cell-Penetrating Peptides with Heparan Sulfate

Outline

Cell-penetrating peptides (CPPs) are able to be taken up by cells and can deliver macromolecular cargos. However, the mechanism of this internalization is not yet fully understood. Recent studies have suggested that negatively charged extracellular glycosaminoglycans (GAGs), such as heparan sulfates (HS), can bind cationic CPPs and form cluster upon these peptides binding to them, thereby affecting the efficiency of a wide range of CPPs. Previously Mihara group has screened the cellular uptake (CU) activities of 54 systematically designed amphiphilic α-helical peptides in HeLa cells and the CU activities of 24 designed peptides have been examined in four cell lines. Notably, even a mutation in a single residue significantly alters the CU ability of a peptide. To determine the structure-CU activity relationship of CPPs, four peptides (RF: GLKKLARLFHKLLKLGC-$_{NH2}$; EF: GLKKLAELFHKLLKLGC-$_{NH2}$; RA: GLKKLARLAHKLLKLGC-$_{NH2}$; EA: GLKKLAELAHKLLKLGC-$_{NH2}$), which contain a difference in one or two amino acid(s) (i.e., Arg/Glu and Ala/Phe), were chosen from the CPP library to examine their interactions with HS in this study.

First, the interactions of the four CPPs with HS were investigated experimentally by means of circular dichroism (CD), fluorescence spectroscopy, isothermal titration calorimetry (ITC), and dynamic light scattering (DLS). Fluorescence spectroscopy and ITC analysis indicated that the HS-binding affinities abilities of the four CPPs correlated well with their CU activities in HeLa and A549 cells (RF ≈ EF > RA > EA), showing that their HS-binding affinities were RF > EF ≈ RA >> EA. The stoichiometry of the binding of CPP to HS leaded to charge neutralization as a molecular principle for binding. The α-helical content of CPP was enhanced when the amphiphilic CPP bound to HS, and the CD analysis yielded an identical order of secondary structure variation of the four designed CPPs in the presence of HS as the order of their HS-binding affinities. Moreover, ITC experiment revealed that their heat capacities were $\Delta C_{P,\mathrm{TAT}}{}^{0} > \Delta C_{P,\mathrm{RA}}{}^{0} > 0 > \Delta C_{P,\mathrm{EF}}{}^{0} > \Delta C_{P,\mathrm{RF}}{}^{0}$, showing that electrostatic interactions were dominant in the HS-binding processes of Arg-containing peptides (RA) in comparison to Glu-containing peptides, whereas hydrophobic contributions were the primary mode of interactions of Phe-containing peptides (EF and RF) in comparison to Ala-containing peptides (RA). DLS analysis showed that the order of the HS-clustering abilities of these CPPs was EF > RF > RA > EA, which also correlated well with their CU activities in HeLa and A549 cells.

Furthermore, the molecular dynamics (MD) simulations in AMBER 12.0 were performed to reveal the detailed binding and clustering mechanisms of these four peptides with HS. The order of the HS-binding affinities and HS-clustering abilities of the four CPPs, which were obtained from the binding free energies calculated in MD simulations ($\Delta G_{RA} \approx \Delta G_{RF} < \Delta G_{EF} < 0 < \Delta G_{EA}$), almost agreed with the experimental data. Using free energy decomposition analyses, it was also indicated a similar result as given by ITC analysis. In comparison to Glu-containing peptides, the Arg-containing peptides were found to contribute more electrostatic energy to the binding enthalpy, whereas the Phe-containing peptides benefited more from non-electrostatic energy in comparison to the Ala-containing peptides. Moreover, although electrostatic interactions were revealed as the initial impetus of HS-CPP binding, non-electrostatic interactions might be more favorable during the HS-CPP clustering process because of their tolerance to local charge mismatch. Consequently, the CPPs which adopted an non-electrostatic dominant HS-binding mechanism, could be internalized by cells more efficiently.

These data, indicating the structure-HS binding affinity relationship of CPPs, will be helpful to identify and design new efficient CPPs for various functions, such as the delivery of macromolecular drugs to cells.