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Cysteine and arginine-rich peptides as molecular carriers

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ABSTRACT

A number of linear and cyclic peptides containing alternative arginine and cysteine residues, namely linear $(CR)_3$, linear $(CR)_4$, linear $(CR)_5$, cyclic $[CR]_4$, and cyclic $[CR]_5$, were synthesized. The peptides were evaluated for their ability to deliver two molecular cargos, fluorescence-labeled cell-impermeable negatively charged phosphopeptide (F′-GpYEEI) and fluorescence-labeled lamivudine (F′-3TC), intracellularly in human leukemia cancer (CCRF-CEM) cells. We investigated the role of cyclization and the number of amino acids in improving the transporting ability of the peptides. The flow cytometry studies suggested that the synthesized peptides were able to work efficiently as transporters for both cargos. Among all compounds, cyclic $[CR]_4$ was found to be the most efficient peptide in transporting the cargo into cells. For instance, the cellular uptake of F′-3TC (5 μM) and F′-GpYEEI (5 μM) was enhanced by 16- and 20-fold, respectively, in the presence of cyclic $[CR]_4$ compared to that of the parent compound alone. The mechanism of F′-GpYEEI uptake by cells was found to be energy-independent. The results showed that the number of amino acids and their cyclic nature can impact the efficiency of the peptide in transporting the molecular cargos.

The cell membrane is composed of a phospholipid bilayer.[1] The bilayer forms an efficient barrier system between intracellular and extracellular aqueous regions. Besides phospholipids, there are also other components present in the structure of the plasma membranes including glycolipids and cholesterol. Several proteins in phospholipid bilayer are responsible for selective transporting of cargos. Moreover, the flexibility of the cell membrane is handled through an interaction of double bonds and free fatty acid chains.[2] The intracellular delivery of highly hydrophobic, poorly water-soluble, and negatively-charged compounds is a challenging task because of the presence of negatively-charged phospholipid bilayer.

To enhance the intracellular concentration of cell impermeable compounds, various classes of drug delivery systems (DDS) have been developed and examined during the last two decades.[3] The discovery and development of a new drug molecule are costly and cumbersome. Thus, improving the cellular delivery of known active compounds using DDS to enhance their biological activity and/or offer controlled release or targeted delivery is a subject of major interest.

Long-circulating macromolecular carriers, such as peptide-based molecules, can exploit the 'enhanced permeability and retention' effect on the site of action.[4] Among various classes of peptide-based DDS, cell-penetrating peptides (CPPs) have been broadly used due to their unique properties such as non-viral nature, biocompatibility, and cell permeability into the plasma membrane.[5] The ability of CPPs has been examined for the delivery of various types of cargos including small drugs[6–9] and large-molecule based therapeutics.[10]

Positively charged amino acids such as arginine and lysine are the main residues in the structure of the majority of CPPs. These amino acids provide positive charges on the surface of CPPs and facilitate electrostatic interactions with negatively charged molecules, such as heparin and phospholipids within the cell membrane.

In addition to the ability of CPPs to penetrate into the cell membrane, the loading and release processes of molecular cargos inside the cell are particularly important in the overall functionality of the system. Thus, researchers tend to avoid obstacles in loading and release processes by taking advantage of non-covalent approaches. This method offers simple loading procedure and smooth intracellular release of the intact cargo.[11,12]

Technically, the sequence of amino acids can modify the molecular interactions, such as electrostatic and hydrophobic interactions between the cargo and the peptide. An optimized orientation could improve the loading capacity of the system by providing appropriate regions to entrap the molecular cargos.

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Thus, a new arrangement of amino acids in peptide-cargo complex can have an impact on the cell permeability and enhance the drug uptake by cells.

Previously, we have reported the synthesis and evaluation of a class of homochiral cyclic peptides with arginine and tryptophan namely $[WR]_4$ and $[WR]_5$ as nuclear-targeting CPPs.[13] These peptides and peptide-capped gold nanoparticles showed a significant efficiency to transport different cargos into cells.[14,15] Our investigations revealed that the existence of a balance between hydrophobic and positive charge properties of peptides was critical for their function as molecular transporters and DDS. Cyclic peptides also offer higher stability in serum compared to the corresponding linear ones.[16]

Cysteine-rich peptides have been previous used as cellular delivery agents.[17] It has been previously reported that a cysteine-rich decapeptide, named CyLoP-1 (Cytosol Localizing Peptide-1) was able to cross the cell membrane readily. This cysteine-rich peptide exhibited the potential for cytoplasmic distribution besides vesicular localization and proficient cellular uptake at low-micro-molar concentrations.[17a] Thus, cysteine appears to contribute in peptide cell permeability.

Herein, we report the preparation of cyclic and linear peptides containing an alternative sequence of cysteine and arginine residues and their evaluation as intracellular molecular transporters. We hypothesized that the presence of cysteine and arginine residues would provide an appropriate combination of amino acids for facilitating the cell permeability of the cargo molecules.

Five cyclic and linear peptides containing alternative sequence of cysteine and arginine amino acids namely *c*[CR]$_4$, *c*[CR]$_5$, *l*(CR)$_3$, *l*(CR)$_4$, and *l*(CR)$_5$ (Fig. 1) were synthesized. Brackets and parenthesis show cyclic and linear peptides, respectively. The peptide synthesis was performed employing 9-fluorenylmethyl oxycarbonyl (Fmoc)-based chemistry according to our previously reported procedure.[13,18]

The synthesis procedure of linear *l*(CR)$_5$ and *c*[CR]$_5$ is shown in Scheme 1 as representative examples. The linear peptide containing ten alternative cysteine and arginine amino acids (CRCRCRCRCR) was assembled on the heterogeneous H-Arg(Pbf)-2-chlorotrityl resin. The Fmoc group at the *N*-terminal of the last coupled amino acid was deprotected using piperidine in DMF (20% v/v) followed by capping using acetic anhydride. The resin was washed and completely cleaved in the presence of reagent R

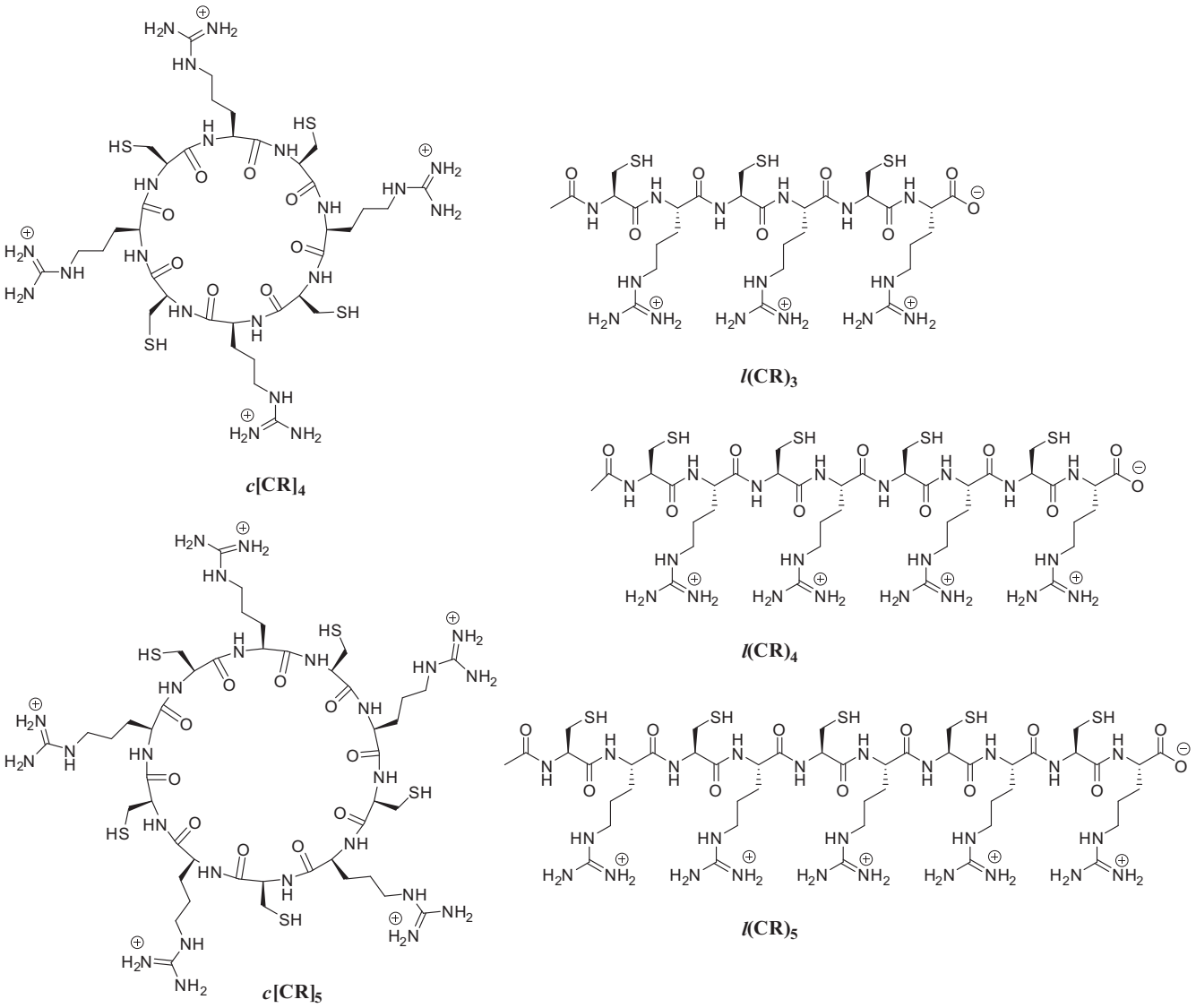

Figure 1. Chemical structures of synthesized peptides.