https://doi.org/10.17952/37EPS.2024.P1225

Development of novel amphipathic stapled peptides as DDS carriers for intracellular delivery of nucleic acids

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01. Introduction

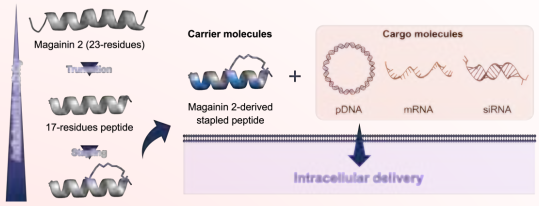
Messenger RNA (mRNA) has no risk of insertion into the host genome, and can express any encoded protein in the cell. Furthermore, small interfering RNA (siRNA) can target genes that cannot be targeted by conventional small molecule drugs and can suppress gene expression in a sequence-dependent manner through RNA interference. Therefore, nucleic acids such as mRNA and siRNA are expected to be next-generation modalities. However, nucleic acids are difficult to permeate cell membranes by themselves due to their high hydrophilicity and large molecular size. Therefore, it is important to develop drug delivery system (DDS) carriers that transport nucleic acids into cells, and cell-penetrating peptides (CPPs) have attracted attention as one of promising carriers.

	pDNA	mRNA	siRNA
Structure	Circular, Double-stranded DNA	Single-stranded RNA	Double-stranded RNA
Base pair	2~200K	~100	20~25
Function	–	Protein expression	mRNA degradation

02. Purpose

We have focused on magainin 2 (**Mag2**), a naturally occurring amphipathic antimicrobial peptide (AMP), and have developed helix-stabilized **Mag2** derivatives containing non-proteinogenic amino acids.[1,2] Because AMPs are capable of intracellular trafficking, they have potential applications as intracellular transport molecules for nucleic acids. In this study, we aimed to develop novel CPPs based on **Mag2** that efficiently transports several types of nucleic acids, such as pDNA, mRNA, and siRNA, into the cell.

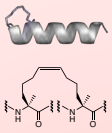
1) M. Hirano *et al.*, *ChemPlusChem* **2020**, *85*, 2731-2736; 2) M. Hirano *et al.*, *Molecules* **2021**, *26*, 444.

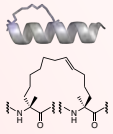


03. Design and Synthesis of Peptide

S_5 = (*S*)-2-(4-Pentenyl)alanine, R_8 = (*R*)-2-(7-Octenyl)alanine

Peptide	Sequence	Peptide	Sequence
Mag2	H-GIGKFLHSAKKFGKAFVGEIMNS-NH$_2$	**st7-1**	H-R_8^*IKKFLKS_5^*AKKFVKAFK-NH$_2$
Pep-1	H-GIKKFLKSAKKFVKAFK-NH$_2$	**st7-2**	H-GR_8^*KKFLKSS_5^*KKFVKAFK-NH$_2$
st4-1	H-S_5^*IKKS_5^*LKSAKKFVKAFK-NH$_2$	**st7-3**	H-GIKKR_8^*LKSAKKS_5^*VKAFK-NH$_2$
st4-2	H-GS_5^*KKFS_5^*KSAKKFVKAFK-NH$_2$	**st7-4**	H-GIKKFR_8^*KSAKKFS_5^*KAFK-NH$_2$
st4-3	H-GIKKS_5^*LKSS_5^*KKFVKAFK-NH$_2$	**st7-5**	H-GIKKFLKR_8^*AKKFVKS_5^*FK-NH$_2$
st4-4	H-GIKKFLKS_5^*AKKS_5^*VKAFK-NH$_2$	**st7-6**	H-GIKKFLKSR_8^*KKFVKAS_5^*K-NH$_2$
st4-5	H-GIKKFLKSS_5^*KKFS_5^*KAFK-NH$_2$	**Pep-1_R**	H-GIRRFLRSARRFVRAFR-NH$_2$
st4-6	H-GIKKFLKSAKKS_5^*VKAS_5^*K-NH$_2$	**st7-5_R**	H-GIRRFLRR_8^*ARRFVRS_5^*FR-NH$_2$

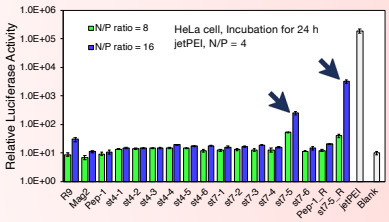




04. Intracellular Delivery of plasmid DNA (pDNA)

N/P ratio: Ratio of positive charge of peptide side chain to negative charge derived from nucleic acid.

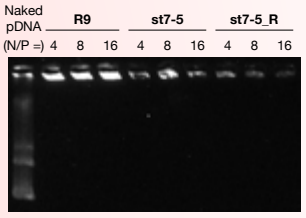
(A) Transfection efficiency of pDNA



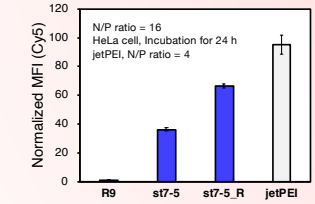
(B) The peptide/pDNA complex size and zeta-potential, gel shift

Peptide	N/P	Size (nm)	PDI (μ/Γ^2)	Zeta-potential (mV)
R9	8	357.5 ± 0.3	0.21 ± 0.01	22.1 ± 0.3
	16	188.7 ± 0.7	0.19 ± 0.01	25.8 ± 0.2
st7-5	8	1522.0 ± 39.0	0.61 ± 0.02	23.4 ± 0.1
	16	234.0 ± 2.1	0.27 ± 0.02	25.6 ± 0.1
st7-5_R	8	352.2 ± 4.1	0.28 ± 0.00	22.3 ± 0.3
	16	125.1 ± 0.2	0.20 ± 0.01	27.0 ± 0.3

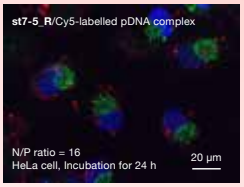
✓ The stapled peptide **st7-5_R** most efficiently introduced pDNA into the cell.
✓ **st7-5_R** formed the smallest complex with pDNA.



(C) Intracellular uptake of Cy5-labelled pDNA

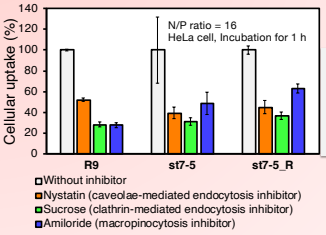


(D) Fluorescence microscopy images



Red: Cy5-laballed pDNA, Blue: Nuclear, Green: Lysosome

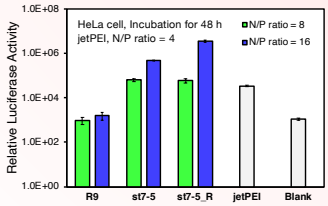
(E) Intracellular uptake pathway of complex



✓ The **st7-5_R**/pDNA complex was efficiently taken up into the cells.
✓ The **st7-5_R**/pDNA complex was suggested to enter the cell via multiple endocytosis pathways.

05. Intracellular Delivery of mRNA

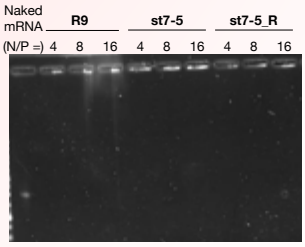
(A) Transfection efficiency of mRNA



(B) The peptide/mRNA complex size and zeta-potential, gel shift

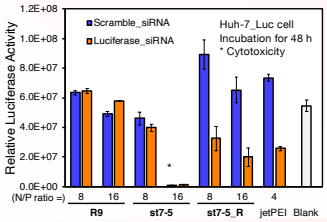
Peptide	N/P	Size (nm)	PDI (μ/Γ^2)	Zeta-potential (mV)
R9	8	257.0 ± 2.5	0.28 ± 0.04	17.3 ± 2.2
	16	177.7 ± 28.8	0.48 ± 0.04	23.5 ± 3.4
st7-5	8	380.4 ± 16.2	0.35 ± 0.01	18.4 ± 0.6
	16	211.3 ± 3.2	0.39 ± 0.07	12.8 ± 1.8
st7-5_R	8	259.3 ± 13.1	0.39 ± 0.03	21.6 ± 1.3
	16	158.3 ± 47.4	0.50 ± 0.10	15.3 ± 1.6

✓ **st7-5_R** transported mRNA into the cell more efficiently than other peptides.
✓ **st7-5_R** formed a small complex with mRNA.



06. Intracellular Delivery of siRNA

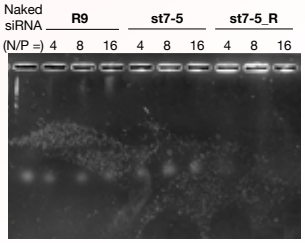
(A) Transfection efficiency of siRNA



(B) The peptide/siRNA complex size and zeta-potential, gel shift

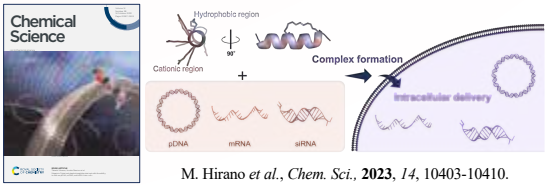
Peptide	N/P	Size (nm)	PDI (μ/Γ^2)	Zeta-potential (mV)
R9	8	849.8 ± 6.5	0.35 ± 0.04	25.6 ± 0.3
	16	690.6 ± 8.7	0.20 ± 0.01	27.9 ± 0.7
st7-5	8	320.0 ± 4.7	0.34 ± 0.03	18.9 ± 0.5
	16	284.8 ± 21.1	0.43 ± 0.00	21.7 ± 1.1
st7-5_R	8	290.4 ± 6.7	0.33 ± 0.01	27.9 ± 1.1
	16	304.0 ± 5.7	0.42 ± 0.04	27.5 ± 2.1

✓ **st7-5_R** delivered siRNA into the cell and suppressed luciferase expression.
✓ **st7-5_R** formed a small complex with siRNA.



07. Summary

- We designed and synthesized stapled peptides based on **Mag2** for the intracellular delivery of nucleic acids.
- The stapled peptide **st7-5_R** formed a small complex with pDNA and achieved efficient intracellular transport.
- The **st7-5_R**/pDNA complex was internalized into the cell by multiple endocytic pathways, independent of any specific pathway.
- **st7-5_R** also formed complexes with mRNA and siRNA to achieve their efficient intracellular transport.



M. Hirano *et al.*, *Chem. Sci.*, **2023**, *14*, 10403-10410.