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Amphipathic short helix-stabilized peptides with cell-membrane penetrating ability

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ABSTRACT

We synthesized four types of arginine-based amphipathic nonapeptides, including two homochiral peptides, R-(L-Arg-L-Arg-Aib)$_3$-NH$_2$ (R = 6-FAM-β-Ala: **FAM-1**; R = Ac: **Ac-1**) and R-(D-Arg-D-Arg-Aib)$_3$-NH$_2$ (R = 6-FAM-β-Ala: *ent*-**FAM-1**; R = Ac: *ent*-**Ac-1**); a heterochiral peptide, R-(L-Arg-D-Arg-Aib)$_3$-NH$_2$ (R = 6-FAM-β-Ala: **FAM-2**; R = Ac: **Ac-2**); and a racemic mixture of diastereomeric peptides, R-(*rac*-Arg-*rac*-Arg-Aib)$_3$-NH$_2$ (R = 6-FAM-β-Ala: **FAM-3**; R = Ac: **Ac-3**), and then investigated the relationship between their secondary structures and their ability to pass through cell membranes. Peptides **1** and *ent*-**1** formed stable one-handed α-helical structures and were more effective at penetrating HeLa cells than the non-helical peptides **2** and **3**.

1. Introduction

Helices in peptides and proteins play an important role in a variety of fields such as biology, chemistry, and medicinal chemistry; therefore, the development of helical peptide foldamers has received increasing attention in recent years.[1] Non-proteinogenic amino acids, such as α,α-disubstituted α-amino acids (dAA) and cyclic β-amino acids, are often utilized as templates for the helical stabilization of short peptides.[2] In particular, α-aminoisobutyric acid (Aib) is the simplest achiral dAA, and has been found to be a useful helical promoter.[3] We have recently reported that the insertion of achiral Aib residues into short Leu-based homochiral L-peptides and heterochiral LD-peptides with alternating L-Leu and D-Leu residues is useful for controlling their helical structures.[4] That is to say, the nona-L-peptide Boc-(L-Leu-L-Leu-Aib)$_3$-OMe (**A**) formed a stable right-handed (*P*) 3_{10}-helix,[4b] whereas the nona-LD-peptide Boc-(L-Leu-D-Leu-Aib)$_3$-OMe (**B**) preferred a (*P*) α-helix (Fig. 1).[4c,d]

Among helical peptides, some amphipathic peptides containing hydrophobic and cationic amino acid residues have unique properties such as antimicrobial activity and the ability to enter cells.[5] Cell-penetrating peptides (CPP) are capable of efficiently delivering hydrophilic molecules, such as peptides, proteins, and DNA, into cells.[6] The hydrophobic amino acid residues and cationic amino acid residues that comprise the helices found in amphipathic CPP are usually aligned in the direction specified for the helix itself. However, natural CPP are not sufficiently stable in the bloodstream as they are susceptible to degradation by proteases. Conversely, non-natural peptides containing D-amino acids and dAA would be expected to have stable helices and be resistant to proteolytic degradation.[5c,7] In addition, stabilized helical amphipathic peptides containing non-proteinogenic amino acids are known to be capable of passing through cellular membranes. As part of our ongoing research into controlling the secondary structures of short peptides, we designed four types of amphipathic nonapeptides containing L-Arg, D-Arg, and achiral Aib residues. Namely, two homochiral peptides, R-(L-Arg-L-Arg-Aib)$_3$-NH$_2$ (R = 6-FAM-β-Ala: **FAM-1**; R = acetyl group (**Ac**): **Ac-1**; FAM: fluorescein) and R-(D-Arg-D-Arg-Aib)$_3$-NH$_2$ (R = 6-FAM-β-Ala: *ent*-**FAM-1**; R = Ac: *ent*-**Ac-1**); a heterochiral peptide, R-(L-Arg-D-Arg-Aib)$_3$-NH$_2$ (R = 6-FAM-β-Ala: **FAM-2**;R = Ac: **Ac-2**); and a racemic mixture of diastereomeric peptides, R-(*rac*-Arg-*rac*-Arg-Aib)$_3$-NH$_2$ (R = 6-FAM-β-Ala: **FAM-3**; R = Ac: **Ac-3**), were synthesized and then the relationship between their secondary structures and cell-penetrating ability was investigated.

2. Results and discussion

2.1. Synthesis of peptides

The **FAM-1**–**3** and *ent*-**FAM-1** peptides, which contained N-terminal fluorescein (6-FAM) labels, as well as N-terminal acetylated

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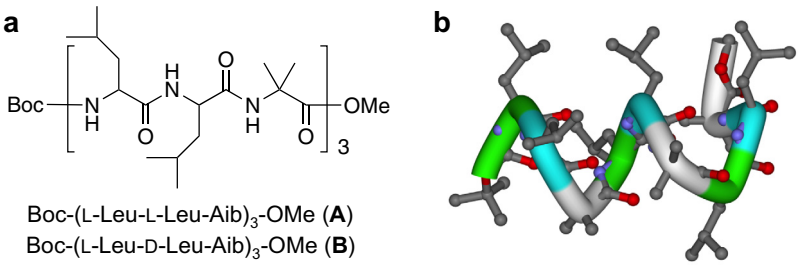

Figure 1. (a) Chemical structures of the nona-L-peptide **A** and the nona-LD-peptide **B**. (b) X-ray diffraction structure of **B**.

Ac-1–3 and *ent*-**Ac-1** peptides were synthesized using a microwave-assisted solid-phase method based on Fmoc protection of their main chain amino groups. All of the peptides were purified by reversed-phase high performance liquid chromatography and characterized by matrix-assisted laser desorption/ionization mass spectrometry (Fig. 2).

2.2. Biological evaluation

We examined the ability of **FAM-1–3** and *ent*-**FAM-1** to pass through the membranes of HeLa cells. After incubating the cells for 2 h at 37 °C, the fluorescence intensity of the resultant cell lysate was measured with a spectrofluorometer. The homochiral

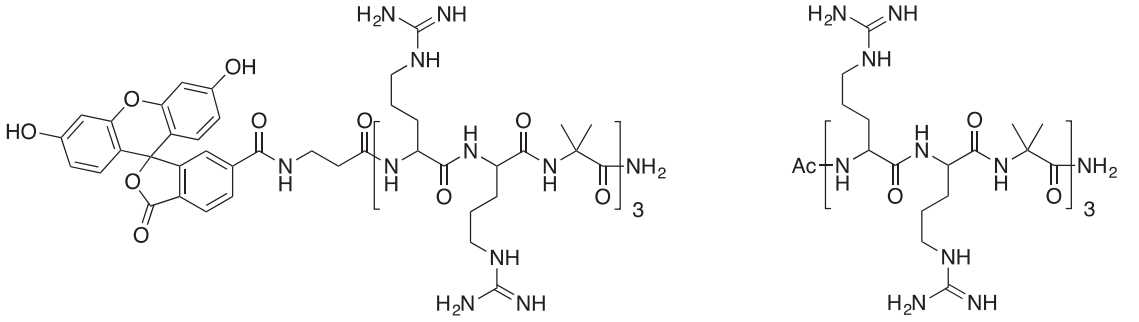

Figure 2. Chemical structures of the designated peptides.

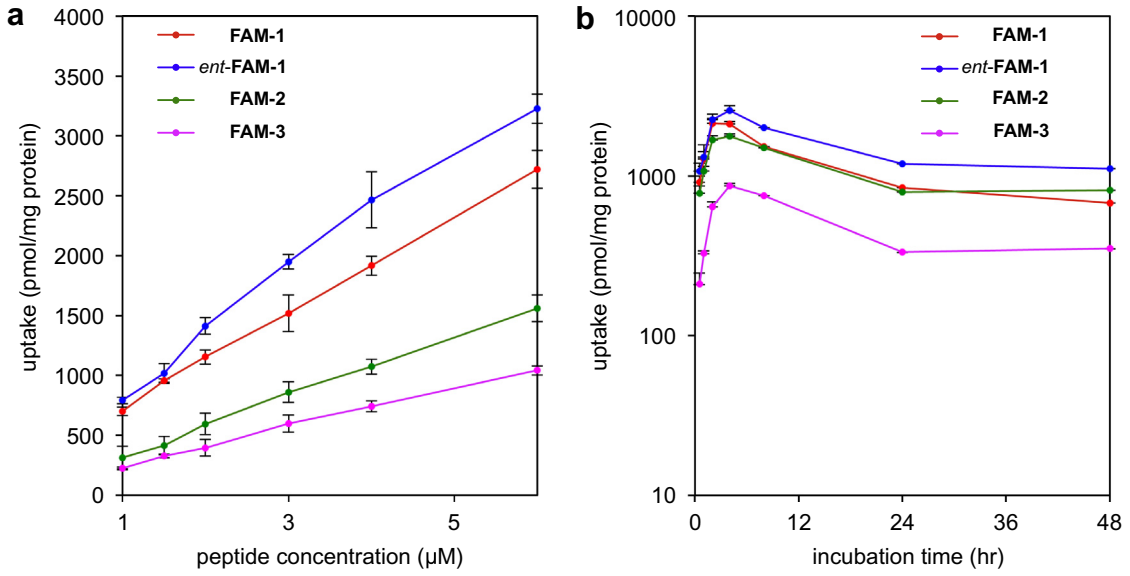

Figure 3. Cellular uptake of **FAM-1–3** and *ent*-**FAM-1** (a) at concentrations of 1–6 μM (incubation time: 2 h), and (b) for incubation periods of 0.5–48 h (peptide concentration: 1 μM). The error bars represent standard deviation, $n = 4$.