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Investigation of the Effect of Aromatic Molecules on the Cell Penetration of Arginine-Rich CPPs

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Introduction

Arginine-rich cell-penetrating peptides (CPPs) are able to transport different cargos into cells, and they are promising subjects of chemical modifications to enhance their cellular uptake efficiency [1]. It was shown by our research group that coupling 4-((4-(dimethylamino)phenyl)azo)benzoyl group (Dabcyl) to tetra- or hexaarginine increases the cell penetration compared to the acetylated peptides [2]. As the Dabcyl group has two aromatic rings, our research group is focused on finding other aromatic molecules with the same penetration enhancing effect as Dabcyl. We investigate the effect of Dabcyl and other aromatic molecules, furthermore aromatic non-natural amino acids on the cellular uptake efficiency of oligoarginine and penetratin derivatives.

Results and Discussion

Our aim is to study the effect of aromatic residues on the intracellular delivery efficiency of arginine-rich CPPs. Therefore, we planned the synthesis of oligoarginines with different number of arginines in their sequence and also penetratin derivatives. The peptides were synthesized with SPPS using Fmoc/^{t}Bu strategy on Rink Amide MBHA resin, and we modified the peptides with various aromatic groups: Dabcyl, 4-(aminomethyl)benzoic acid (AMBA) (Figure 1) and (*E*)-3-(4-hydroxyphenyl)-2-phenylacrylic acid (cis-stilbene derivative), 3-(2-naphthyl)-L-alanine and L-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid. The fluorescent dye 5(6)-carboxyfluorescein was coupled either to the side chain of an inserted *C*- terminal Lys in solution, or to the *N*-terminal α-amino group of the peptide on solid phase. The fluorescence intensity of EBC-1 cells treated with the conjugates for 90 minutes at room temperature was measured by flow cytometry.

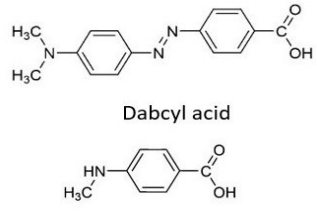


Fig. 1. The structure of Dabcyl acid and AMBA.

In case of the prepared oligoarginine derivatives (from four arginines to eight arginines in their sequence) the Dabcyl group was an effective penetration enhancer, the AMBA modification caused no change in the cellular uptake, and usually the Dabcyl-AMBA tandem group was better than Dabcyl alone (Figure 2). The position of the Dabcyl and 5(6)-carboxyfluorescein was important only in case of one conjugate, the hexaarginine derivative Dabcyl-Arg$_6$-Lys(Cf), where the Cf-Arg$_6$-Lys(Dabcyl) seemed to be a poorer CPP than octaarginine. The results from the concentration-dependent uptake of selected oligoarginine derivatives clearly showed that Dabcyl-AMBA-Arg$_8$-Lys(Cf) is a prominent CPP even at 1.25 μM concentration. Also, Dabcyl-Arg$_6$-Lys(Cf) and Dabcyl-AMBA-Arg$_5$-Lys(Cf) have as similar uptake efficiencies as octaarginine. Regarding the uptake mechanisms, the Dabcyl-AMBA-Arg$_8$-Lys(Cf) peptide enters cells by macropinocytosis, while the Dabcyl-Arg$_6$-Lys(Cf) and Dabcyl-AMBA-Arg$_5$-Lys(Cf) peptides are uptaken mostly by lipid-raft/caveolae-dependent endocytosis, but pinocytosis is also a route for them.

The peptides modified with (*E*)-3-(4-hydroxyphenyl)-2-phenylacrylic acid (St), St.-Arg$_4$-Lys(Cf) and St.-Arg$_2$-Trp-Arg$_2$-Lys(Cf) showed poor cellular uptake, we think it might be the cause of the *E*- conformation (results not shown).

Based on the results of Letoha et al. [3] who showed that dodeca-penetratin can be equally effective as penetratin, we synthesized penetratin and dodeca-penetratin derivatives, and changed the two tryphtophans and the phenylalanine in the sequence either to 3-(2-naphthyl)-L-alanine (Nal) or to 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (TIC) (Table 1 and Figure 3). Later Cf-Pen12 was further modified with Dabcyl and/or Nal (Table 1). The result (Figure 4) showed that the Nal modification has a significant enhancing effect on the cellular uptake, and in this case the shortened peptide can be used on EBC-1 cells. The cell penetration efficiencies of the TIC modified peptides are

greatly reduced compared to that of the the unmodified peptides. Probably the TIC caused structural disturbance is the casue of this negative effect. From the results of the uptake of the Dabcyl and/or Nal modified peptides we can conclude that Dabcyl can also enhance the internalisation of penetratin, but it is better to use it alone than in combination with Nal modifications.

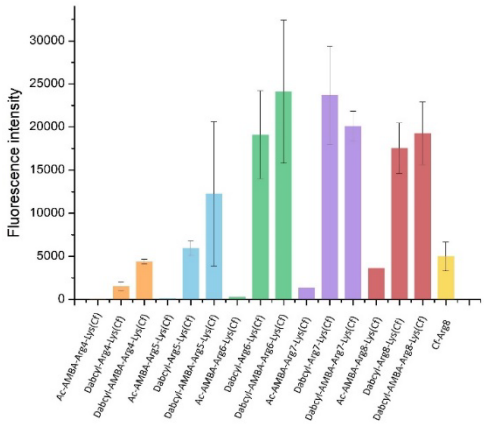
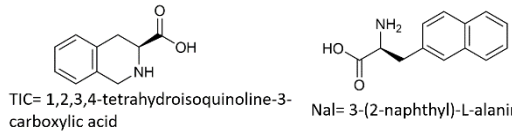


Fig. 2. Internalisation of conjugates into EBC-1 cells at 5 μM concentration at RT.

Table 1. Sequence of synthesized penetratin derivatives.

Abbreviation	Sequence
Cf-Pen	Cf-RQIKIWFQNRRKWKK-NH_2
Cf-Pen(Tic)	Cf-RQIKI*TICTIC* QNRRK*TIC* KK-NH_2
Cf-Pen(Nal)	Cf-RQIKI*NalNal* QNRRK*Nal* KK-NH_2
Cf-Pen12	Cf-RQIKIWFRKWKK-NH_2
Cf-Pen12(Tic)	Cf-RQIKI*TICTIC* RK*TIC* KK-NH_2
Cf-Pen12(Nal)	Cf-RQIKI*NalNal* RK*Nal* KK-NH_2
Ac-Pen12(Cf)	Ac-RQIKIWFRKWKK(Cf)-NH_2
Ac-Pen12(Nal)(Cf)	Ac-RQIKI*NalNal* RK*Nal* KK(Cf)-NH_2
Dabcyl-Pen12(Cf)	Dabcyl-RQIKIWFRKWKK(Cf)-NH_2
Dabcyl-Pen12(Nal)(Cf)	Dabcyl-RQIKI*NalNal* RK*Nal* KK(Cf)-NH_2

Fig. 3. The structure of TIC and Nal.



TIC= 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid

Nal= 3-(2-naphthyl)-L-alanine

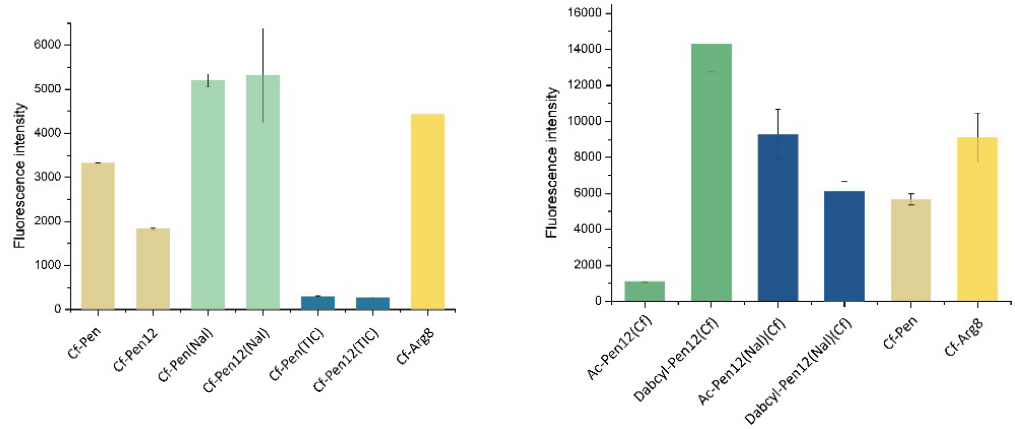


Fig. 4. Internalisation of penetratin derivatives and internal standard Cf-Arg_8 into EBC-1 cells at 5 μM concentration at RT.

Acknowledgments

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