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CHARACTERIZATION OF PTH-CPP FUSION PEPTIDES FOR ORAL DELIVERY

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Introduction

Peptides and proteins play a variety of important roles for diverse biological functions and due to an extensive progress in the biotechnological area it is now possible to produce a high number of potential therapeutic peptide and protein drugs. Oral dosage forms are preferred when taking patient compliance into consideration; however, oral delivery of peptide and protein drugs constitutes a number of challenges due to the large molecular size, enzymatic instability and poor membrane permeability of the molecules. To overcome the poor membrane permeability, the use of cell penetrating peptides (CPP's) are of great interest [1] as they have shown potential in improving the transepithelial transport of therapeutic peptides upon co-administration [2] or direct conjugation [3]. Of these two methodologies, co-administration is generally preferred due to a possibly better protection of the biological activity of the drug and ease in sample preparation, whereas a drawback is the possible generation of poorly characterized complexes when simply mixing the CPPs with the therapeutic peptides.

Knowledge is however lacking regarding the effect on the biological activity of the therapeutic peptide or protein and the cell penetrating propensity of the CPP as a result of direct conjugation; an issue addressed in the present study. Hence, the objective of this study is to produce a number of fusion peptides comprising the biologically active part of parathyroid hormone (PTH1-34) coupled to the CPPs penetratin (Pen), the HIV-1 Transactivating (Tat) protein, the HSV-1 structural protein VP22 or nonaarginine (R9). These fusion peptides have been characterized according to secondary structure, which is thought to be relevant for the potency of PTH1-34 and the cell penetrating propensity of the CPPs [4].

Results and Discussion

PTH1-34 and 7 CPP-coupled PTH1-34 (Fig. 1) fusion peptides have been expressed and purified employing a newly developed protocol for the expression and purification of insoluble inclusion bodies (IBs). Briefly, DNA constructs were cloned into a His-tag vector using ligase-independent cloning and expressed in *E. coli* as IBs. The IBs were solubilized in 6M guanidinium and subjected to affinity chromatography followed by reverse phase (RP) HPLC for purification. The His-tag was removed by incubation with a TEV protease (Fig. 2a) and separated from the fusion peptides by RP-HPLC (Fig. 2b). Molecular weights were verified by mass spectrometry (MS) (Fig. 2c).



Figure 1. Model illustrating the N- and C-terminally CPP-coupled PTH1-34 fusion peptides, which are expressed and purified from IBs.

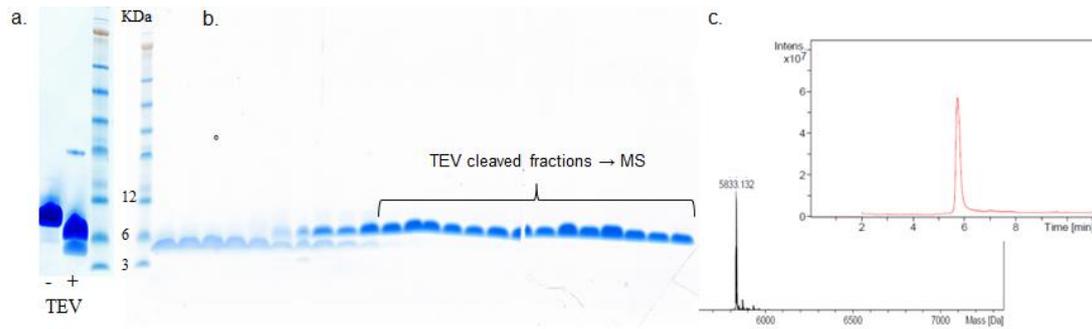


Figure 2. Representative purification of Tat-PTH1-34 after cleavage of the His-tag (a), separation of the His-tag from Tat-PTH1-34 by RP-HPLC (b) and verification of the molecular weight by MS (c).

The secondary structure of PTH1-34 and the PTH1-34 fusion peptides was analysed by circular dichroism (CD) spectroscopy (Fig. 3) in the absence or presence of trifluoroethanol (TFE), which is widely used as an α -helix promoting agent. Without TFE, PTH1-34 and the fusion peptides lacked secondary structure (Fig. 3a), but with TFE the α -helical content increased as observed by a shift towards minima at 208 nm and 222 nm (Fig. 3b). Whether the CPP was N- or C-terminally coupled to PTH1-34, only affected the spectra of the Tat conjugated PTH1-34 when TFE was present. This indicates that the specific N- or C-terminal positioning of Tat might affect the potency of PTH1-34 and the cell penetrating propensity of Tat, as an α -helical content is important for receptor binding and interaction with the plasma membrane, respectively.

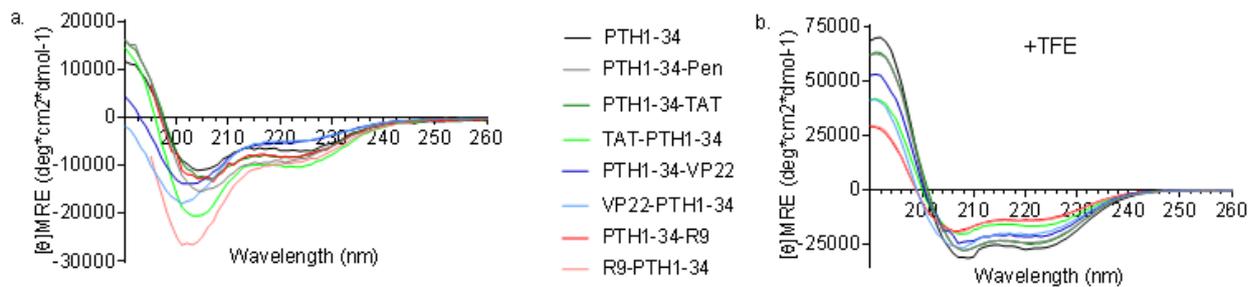


Figure 3. CD spectra of 10 μ M PTH1-34 or 10 μ M CPP-modified PTH1-34 fusion peptides in the absence (a) or presence (right) of 20% TFE.

Conclusion

PTH1-34 and N- or C-terminally CPP-coupled PTH1-34 fusion peptides were successfully expressed and purified from IBs. CD spectra revealed a disordered secondary structure in buffer, but with TFE added the α -helical content increased in all peptides. A difference in α -helical content as a result of N- or C-terminal coupling of CPP was only observed for the Tat-coupled PTH1-34 indicating that the specific N- or C-terminal positioning of Tat might be of relevance for membrane perturbation and receptor activation.

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