

P002 Peptide degradation is a critical determinant for cell-penetrating peptide uptake.

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Cell-penetrating peptide mediated uptake of labels appears to follow an equilibrium-like process. However, this assumption is only valid if the peptides are stable. Hence, in this study we investigate intracellular (IC) and extracellular (EC) peptide degradation kinetics of two fluorescein labeled cell-penetrating peptides, namely MAP and penetratin, in Chinese hamster ovarian cells. The degradation and uptake kinetics were assessed by RP-HPLC equipped with a fluorescence detector. We show that penetratin and MAP are rapidly degraded both extracellularly and intracellularly giving rise to several degradation products. Kinetics indicates that intracellularly, the peptides exist in (at least) two distinct pools: one that is immediately degraded and one that is stable, suggesting that two processes are involved. One mechanism that gives rise to N-terminally truncated IC peptides, which is in accordance with earlier studies, and another mechanism which is mainly responsible for the intact IC peptide. Moreover, the degradation could be decreased by treating the peptides with BSA or phenanthroline and the uptake was significantly reduced by cytochalasin B, chloroquine and energy depletion. The results indicate that the EC degradation determines the IC peptide concentration in this system and therefore the stability of cell-penetrating peptides needs to be evaluated.