Lasso Peptides

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Introducing Lasso Peptides as Molecular Scaffolds for Drug Design: Engineering of an Integrin Antagonist**

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Peptides combine a high specificity for their target receptor with a low toxicity and are therefore a promising source for drug leads.[1,2] However, their use has been limited because of undesirable physicochemical and pharmacokinetic properties.[2] To overcome these obstacles protein scaffolds, such as ultrastable ribosomally assembled peptides, can be used together with synthetic chemical strategies to present biologically active peptide epitopes, as shown recently by the conversion of the cyclotide kalata B1 into a vascular endothelial growth-factor-A antagonist.[3–7] In addition, bacterial lasso peptides have been under discussion as molecular scaffolds for drug design.[8–10] These ribosomally assembled peptides consist of 16–21 amino acids and share an N-terminal eight/nine-residue macrolactam ring through which the C-terminal linear tail is threaded and trapped by steric hindrance of bulky side chains.[11–15]

The currently known gene clusters of the lasso peptides microcin J25 (MccJ25) and capistruin consist of four genes, one coding for the precursor protein, two for the processing enzymes, and one for the export and immunity protein.[15,16] Mutational analysis of the precursor proteins of MccJ25 and capistruin revealed a high promiscuity of the biosynthetic machineries and the feasible heterologous production of various variants in *Escherichia coli*.[9,10] Therefore, lasso peptides combine unique characteristics relevant for their application as robust scaffolds for epitope grafting (Figure 1): 1) extraordinary stability against proteolytic degradation, temperature, and chemical denaturants; 2) gene-encoded lasso peptide precursor proteins; 3) a gene cluster of bacterial origin allowing heterologous production in *E. coli*; and 4) a promiscuous biosynthetic machinery tolerating various amino acid substitutions within the lasso peptide sequence.[9–11,15]

To prove their applicability as molecular scaffolds, we chose the integrin binding motif RGD as peptide epitope to be grafted onto a lasso peptide structure. Integrins are a large class of heterodimeric cell surface receptors and the RGD-binding integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$ have received increasing interest as therapeutic targets because of their role in tumor growth and angiogenesis.[17,18] As an insertion site the turn motif inside the threading tail was chosen to present: 1) the RGD epitope on the surface of the lasso structure to facilitate productive interaction with the binding site between the two integrin subunits; and 2) a conformationally restricted, kinked geometry responsible for the superior activity and selectivity profiles of cyclic RGD peptides compared to their linear representatives.[19–21] Thus, only MccJ25 was suitable as a molecular scaffold, since the biosynthetic machinery of capistruin does not tolerate substitutions within the noose.[9] Consequently, the tripeptide sequence Gly12-Ile13-Gly14 (numbering according to MccJ25) was substituted by Arg-Gly-Asp through site-directed mutagenesis of the precursor protein McjA, which is encoded alongside the processing enzymes McjB/McjC and the export and immunity protein McjD on the pTUC202 plasmid (Figure 1 in the Supporting Information).[16] HPLC–HRMS analysis of the culture supernatant of *E. coli* DH5α harboring the pTUC202 RGD plasmid revealed the successful maturation of the mutated precursor protein into the MccJ25 RGD triple mutant, which could be purified to homogeneity with a yield of 0.7 $mg\,L^{-1}$ (Figure 2 in the Supporting Information), consistent with the processing and production rate of the single mutants.[10] Tandem mass spectrometry (MS^2) fragmentation studies and carboxypeptidase Y digestion assays confirmed the lasso structure of MccJ25 RGD (see the Supporting Information).

The affinity of MccJ25 RGD towards $\alpha_v\beta_3$, $\alpha_v\beta_5$, $\alpha_5\beta_1$, and $\alpha_{IIb}\beta_3$ integrins was analyzed by inhibition assays of integrin–extracellular matrix protein binding. The wild-type peptide MccJ25, the linear heptapeptide P1 (Ac-FVRGDTP-NH_2) corresponding to the sequence of the turn motif in MccJ25 RGD, the cyclic pentapeptide cilengitide (cyclo[RGDf-N(Me)V-]), and the non-peptide α_{IIb} integrin inhibitor tirofiban served as controls.[22–24] MccJ25 did not show biological activity towards $\alpha_v\beta_3$, $\alpha_v\beta_5$, $\alpha_5\beta_1$, and $\alpha_{IIb}\beta_3$ integrins, thus proving the molecular framework to be inactive (Table 1). In contrast, the installation of the RGD epitope in the turn motif of the MccJ25 scaffold resulted in a remarkable increase of its binding affinity, with IC_{50} values of

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