Abstract

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Exploring protein functionalisation – The site-selective modification of designed four-helix bundle scaffolds

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This thesis describes two complementary strategies for the site-selective functionalisation of folded helix-loop-helix dimers in aqueous solution where control of the site selectivity is based exclusively on the reactivity of the naturally occurring amino acids. Access to site-selective acylation reactions make folded proteins excellent supramolecular scaffolds with a wide range of applications in chemistry, biotechnology and biomedicine.

A number of 42-residue polypeptides that fold into helix-loop-helix dimer motifs and that are capable of site-selective self functionalisation have been designed and synthesised to explore the range and the selectivity of the histidine-mediated lysine and ornithine side chain acylation reaction. The hierarchy of reactivities of the lysine residues situated close to the histidine has been determined. A lysine in position $i+4$ relative to a histidine in position i in a helical sequence was previously shown to be the most reactive, but there are at least four other positions on the surface of the folded polypeptide that can be individually addressed via the histidine-mediated pathway. It was also shown that it was possible to selectively acylate the side chain of an ornithine residue without the presence of a histidine. The interactions that, at the molecular level, control the reactivity of lysines and ornithines have therefore been investigated and used in the development of a site-selective acylation strategy that addresses lysine or ornithine residues directly. It was found that it is possible to depress the pK_a values of lysine and ornithine residues by more than one pK_a unit, and thus make them more reactive, by placing them in the microhydrophobic environment of the hydrophobic core of the folded four-helix bundle motif.

In one application of the histidine-mediated functionalisation reaction, two different glycoconjugates were incorporated into a folded polypeptide and their effects on the structure were determined. The glycoconjugate was shown to stabilise the structure of a folded polypeptide with a disordered hydrophobic core but not to affect a peptide with a well-defined tertiary structure.

The direct acylation reaction was used in combination with an orthogonal protecting group strategy in the design and synthesis of a biosensor where strong fluorescence intensity enhancement signalled the specific binding of a protein, human carbonic anhydrase II.

The two functionalisation strategies operate most efficiently at different pH and are therefore complementary. They have been used for the stepwise and pH-controlled introduction of three different functional groups into a helix-loop-helix motif scaffold without any intermediate purification, giving an overall yield of approximately 30% of the correct trisubstituted peptide.

KEYWORDS: *de novo* design, polypeptide, helix-loop-helix, site-selective functionalisation, scaffold, glycopeptide, biosensor

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