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Access to the Inaccessible Sequence of Cpn 60.1 (195–217) by Temporary Oxazolidine Protection of Selected Amide Bonds

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Abstract—The solid-phase peptide synthesis of a reportedly inaccessible peptide sequence of chaperonin 60.1 (195–219) is described using oxazolidine containing dipeptide building blocks ('pseudo-proline' dipeptide units). Two attempts at the synthesis of the chaperonin 60.1 sequence are outlined using one and two pseudo-proline units, respectively, and these results are compared with the outcome of an ordinary stepwise (double) coupling procedure. The only successful synthesis is that combining two pseudo-proline building blocks.

Difficult peptide sequences are those that do not form readily using solid-phase peptide synthesis (SPPS). The reason appears to be low coupling yields caused by aggregation (driven by regions of excessive hydrophobicity in the growing protected polypeptide chain) or secondary structure formation (β-sheets). Low resin loading and/or protection of peptide link nitrogen atoms have been used to prevent the formation of hydrogen bonds that assist both aggregation and secondary structure formation. Fragment condensation[1] or chemoselective ligation procedures[2–5] have proven to be useful alternative approaches to achieving the same goal. The most popular protecting group for peptide link nitrogen atoms is the *N*-(2-hydroxy-4-methoxybenzyl) (Hmb) protecting group (cf. Fig. 1).[6] Hmb-protected amino acids are commercially available, and successful applications have been described.[7,8]

However, low coupling yields and undesirable side products can occur with Hmb-deprotection thereby reducing the general utility of this method.[9] Consequently, other protection methodologies have been sought for including the use of oxazolidine or thiazolidine 'pseudo-proline' dipeptide units.[10] These units serve a dual role both to protect peptide link nitrogen atoms from hydrogen bond formation and also to induce backbone conformations in the growing protected polypeptide chain that are less prone to aggregation. Pseudo-proline dipeptide units (Xaa-Ψpro) are formed through the cyclocondensation of serine, threonine or cysteine side chains onto their respective N-terminal peptide link nitrogen atoms.[11] This cyclocondensation leads to enhanced flexibility about the peptide link owing to the loss of double bond character between carbon and nitrogen atoms with the formation of a tertiary amide.[12,13] The enhanced flexibility is manifested in the quantitative induction of *cis*-Xaa-Ψpro peptide bonds (cf. Fig. 2),[14] that create significant kinks in the polypeptide backbone, thus preventing the formation of aggregated structures. Where comparisons have been made, pseudo-proline dipeptide units have been demonstrated to be superior to Hmb-protecting groups.[15] However, pseudo-proline dipeptide units may be used only for the synthesis of protected peptide sequences harboring serine, threonine or cysteine residues hence somewhat restricting the utility of this method. Nevertheless, a number of pseudo-proline

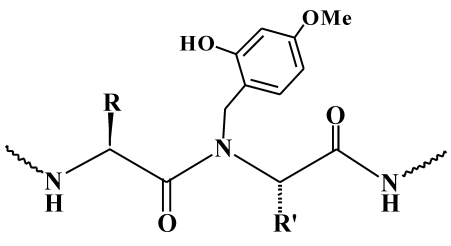

Figure 1. Hmb protecting group within a dipeptide unit.

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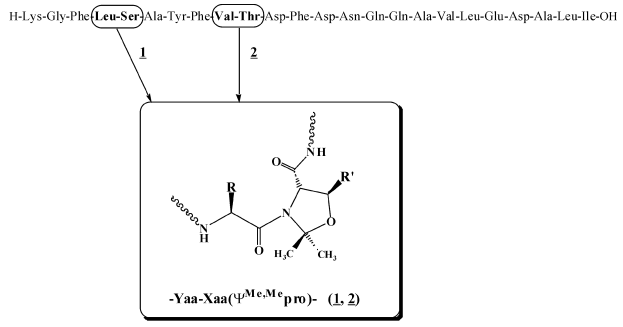

Figure 2. Molecular structures of the oxazolidine building blocks **1** and **2** -[Yaa-Xaa($\Psi^{Me,Me}$pro)]- used for the successful synthesis of chaperonin 60.1 (195–217) sequence. **1**: Yaa; Leu: R = isobutyl, Xaa; Ser: R′ = H. **2**: Yaa: Val; R = isopropyl, Xaa; Thr: R′ = CH_3.

dipeptide units are now commercially available.[16] Here we report the use of the pseudo-proline strategy for the synthesis of a peptide with sequence (195–217) derived from the molecular chaperone protein, chaperonin 60.1 (cf. Fig. 2). Our interest in this chaperonin 60.1 fragment began with recent reports concerning the role of molecular chaperones to induce extracellular IL-1β and IL-6 gene expression in human monocytes by a mechanism independent of chaperone protein conformation.[17] Molecular chaperones are more familiar as proteins that assist the folding of other proteins. However, recent evidence also suggests that chaperonin proteins may be key mediators of the inflammation processes induced by pathogenic microorganisms with infection.[18,19] Enzymatic digestion of chaperonin 60.1, followed by fractionation of the peptide fragments gave one highly biologically active fraction comprised of peptide (195–217), as determined by mass spectrometry. Unfortunately, subsequent attempts to prepare this peptide by SPPS[20] proved unsuccessful. Therefore, we elected to apply the pseudo-proline strategy to try and obtain the desired peptide sequence making use of threonine and serine residues present in the sequence (cf. Fig. 2).

Peptides were synthesised on a fully automated Advanced ChemTech 348 Omega synthesiser using double couplings (30 min) followed by a capping step of acetic anhydride (15 min). The automated procedure eliminates irregularities of manual synthesis, and the outcome, i.e., purity of the crude peptide, is a direct measure of the coupling efficiency in function of the strategy used. Three different peptide synthesis strategies were compared: (a) ordinary double coupling/capping strategy; (b) double coupling using only one oxazolidine pseudo-proline unit, Fmoc-Val-Thr($\Psi^{Me,Me}$pro)-OH **2**; (c) double coupling using two pseudo-proline units, Fmoc-Val-Thr($\Psi^{Me,Me}$pro)-OH **2** and Fmoc-Leu-Ser($\Psi^{Me,Me}$pro)-OH **1**. As the analytical HPLC traces show, only one strategy (c) was successful (cf. Fig. 3). Both strategies (a) and (b) failed, giving two main products eluting at almost equivalent gradient positions. Characterisation of both peaks by electron-spray mass spectrometry revealed one to be the correct product and the other to be missing the N-terminal lysine residue ($m/z = 2575.3$ [M − Lys + H^+]). These were never completely separable by HPLC. This emphasizes how difficult this sequence is to prepare by normal SPPS

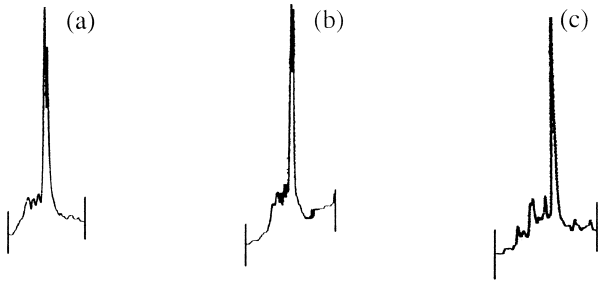

Figure 3. HPLC sections around the retention time of crude chaperonin 60.1 (195–217) peptides. (**a**) Ordinary double coupling/capping strategy yielding a peptide with a failure sequence. (**b**) Insertion of the Val-Thr unit as a pseudo-proline unit **2**. (**c**) Use of two pseudo-proline units (Fmoc-Leu-Ser[$\Psi^{Me,Me}$pro]-OH) and **1** (Fmoc-Val-Thr[$\Psi^{Me,Me}$pro]-OH) **2** affording pure crude peptide. Gradient used for analysis: 0–100% acetonitrile in 20 min.

methods and clearly exemplifies the power of the pseudo-proline unit protecting strategy, particularly if used extensively throughout a growing protected peptide sequence.

In conclusion, it is demonstrated that multiple use of pseudo-proline units as peptide link nitrogen atom protecting groups leads to quantitative coupling yields throughout a difficult peptide sequence. Single use of a pseudo-proline unit was ineffective demonstrating that these units have only a local effect. However, the likelihood is that the inclusion of pseudo-proline units wherever serine, threonine or cysteine residues are located will have a beneficial effect on overall coupling efficiency and hence the overall yield of the synthesis of difficult sequences.

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