A three stranded β-sheet peptide in aqueous solution containing *N*-methyl amino acids to prevent aggregation

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A three stranded β-sheet peptide of *de novo* design with *N*-methyl amino acids to prevent aggregation folds upon heating.

Peptides which fold in isolation provide a useful way to study protein secondary structure and stability. The α-helix has been studied in this way for a number of years since the discovery that *de novo* designed peptides of 15–20 amino acids in length, composed primarily of alanine, form isolated α-helices in water.[1] In contrast to the mature field of α-helical peptides, β-sheet peptides are much less well understood. A challenge is to synthesise a peptide which will fold into a β-sheet in aqueous solution without aggregation. Synthetic polypeptides that form β-sheets generally form large, insoluble structures comprising many polypeptide chains that are intractable for further study in aqueous solution. However, success has been achieved using both peptides containing natural amino acids[2–13] and with β-sheet formation promoted by non-peptide units.[14–20] Here a peptide designed to fold into a three-stranded β-meander is synthesised, and the fact that it folds into a β-sheet is demonstrated by circular dichroism (CD) spectroscopy.

The β-meander, sequence Ac-YGD(*N*-Me-A)A(*N*-Me-A)APGVVDDVVPGD(*N*-Me-A)AVA(*N*-Me-A)-$CONH_2$, was designed to fold into the structure shown in Fig. 1. Aggregation is avoided in two ways. Firstly, negatively charged Asp side chains are located on both faces of the sheet to introduce intermolecular charge repulsions. Secondly, both outer edges of the sheet have two *N*-methylated amino acids. The *N*-methyl bonds are unable to participate in intermolecular hydrogen bonding and hence the peptides will remain monomeric. These residues are also restricted in their backbone conformations to the β-sheet geometry, which further facilitates folding.[21] *N*-Methylation has previously been used to prevent dimerisation of Interleukin-8 by blocking intermolecular hydrogen bonding.[22] Ghadiri *et al*. have *N*-methylated peptide nanotubes on one side so that they form dimers tractable to NMR spectroscopy and X-ray crystallography.[23]

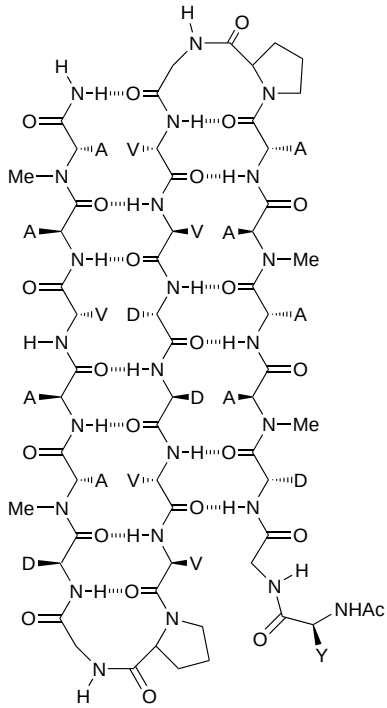

Fig. 1 Design of the three stranded β-meander peptide

The other amino acids in the sheet were chosen as follows: Pro-Gly is the most commonly observed turn sequence in proteins,[24] while valine residues have a strong preference for β-sheets.[25,26] The formation of the N–Me to C=O bond is synthetically challenging.[27] This is generally made easier by forming *N*-Me-Ala to Ala bonds where possible. In particular, β-branched amino acids are avoided preceding *N*-methyl amino acids. The Gly-Tyr sequence is designed to give the peptide a UV absorbance unperturbed by secondary structure for accurate concentration determination.[28]

The syntheses of peptides containing *N*-methyl amino acids or those that form β-sheets are particularly difficult. The peptide designed here is therefore doubly challenging to synthesise. Peptides were synthesised by the solid phase method using Fmoc chemistry. Success was first achieved with Rink resin and Fmoc amino acids and the reagent TBTU [(benzotriazol-1-yloxy)tris(pyrrolidino)phosphonium hexafluorophosphate] in four-fold excess in the presence of Pr^i_2EtN, as judged by analytical fast protein liquid chromatography (FPLC) on the crude yield and confirmed by mass spectrometry. The yield was too low for further analysis so the powerful new reagents, HATU [*O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate] and HOAt (1-hydroxy-7-azabenzotriazole)[29,30] were used with Fmoc-PAL-PEG-PS resin and double coupling cycles for the N–Me to C=O bonds. *N*-Termini were acetylated using pyridine and Ac_2O. Cleavage from the resin was achieved with TFA–anisole (19 : 1). After precipitation of the peptide–TFA mixtures into dry ice-coooled Et_2O, the peptides were purified by C_{18} reverse-phase FPLC (Pharmacia) using a H_2O–MeCN gradient of 10–30% containing 0.1% TFA, followed by size-exclusion chromatography, giving a 10% yield (10 mg) of the target product.

The peptide is soluble in aqueous solution up to at least 2 mM concentration and appears to be monomeric by size exclusion chromatography and 1D NMR line shape analysis (data not shown). CD spectra were acquired at 18 μM concentration, pH 7, 5 mM phosphate buffer, 10 mM NaCl. Fig. 2 shows the CD spectra acquired at 293, 313 and 333 K. At 293 K the peptide is a mixture of random coil and β-sheet. Remarkably, it folds upon heating, giving a classic β-sheet spectrum at 333 K, with a minimum at 219 nm.[31] Hence, β-sheet formation is endothermic. The isodichroic point at 212 nm suggests that the sheet/coil transition is two-state.

It is proposed that *N*-methylation of NH groups on the outer edges of a β-sheet is a general solution to the problem of β-sheet aggregation. The peptide developed here may provide a suitable model system for investigating a range of aspects of β-sheet stability and folding.

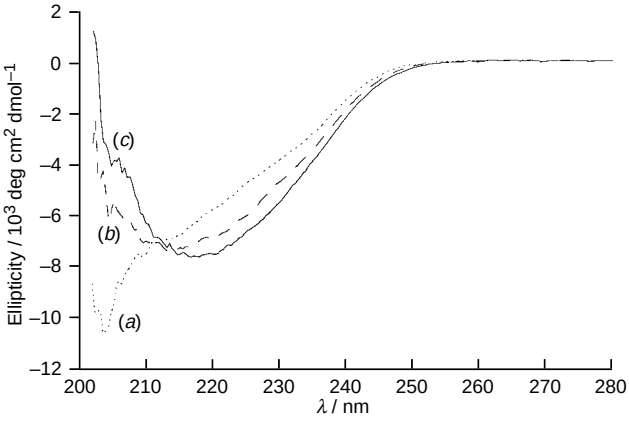

Fig. 2 CD Spectra of the β-meander (pH 7, 5 mM phosphate, 10 mM NaCl) at (*a*) 20, (*b*) 40 and (*c*) 60 °C

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Footnote and References

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