

α and β Conformational preferences in fibril forming peptides characterised using NMR and CD techniques

Thelma A. Pertinhez^{a,b}, Amanda K. Sherwood^a, Leonardo F. Fraceto^c, Mario Bouchard^a, Maureen Pitkeathly^a and Lorna J. Smith^{a,*}

^a *Oxford Centre for Molecular Sciences, Central Chemistry Laboratory, University of Oxford, South Parks Road, Oxford, OX1 3QH, UK*

^b *Center for Molecular and Structural Biology, LNLS, CP 6192, 13084-971, Campinas, Brazil*

^c *LNLS and Department of Biochemistry, University of Campinas, Campinas, Brazil*

Abstract. Peptide fragments taken from residues 18–54 of short consensus repeat 3 (SCR3) from the human complement receptor CR1 have been found in aqueous solution to slowly aggregate and form fibrils. NMR studies of the monomeric form of these peptides show that they are essentially unfolded in aqueous solution and that they all have an increased helicity in TFE solutions. The behaviour of residues 28–31 from SCR3 is particularly interesting. These residues have a high β -sheet propensity in the native protein and a seven peptide containing their sequence is found to form fibrils despite its short length. However, NMR studies show that these residues adopt a well-defined α -helix in 80% TFE and under these conditions fibril formation has not been observed. These data demonstrate the strong dependence of conformational propensities on environment.

Abbreviations

CD, circular dichroism; EM, electron microscopy; SCR3, short consensus repeat 3 from the human complement receptor CR1; SDS, sodium dodecyl sulfate; TFE, trifluoroethanol.

1. Introduction

A number of different proteins have been found to form amyloid fibrils *in vitro*. These include the SH3 domain of PI3 kinase, a type III domain of fibronectin, acylphosphatase, myoglobin and cytochrome *c*₅₅₂ [1–5]. It is therefore becoming increasingly apparent that it is not only the 20 or so disease-associated proteins that can form the highly ordered fibrillar aggregates but rather that the propensity for this type of structure may be an intrinsic, generic property of the polypeptide backbone [6]. Indeed myoglobin and cytochrome *c*₅₅₂ that are substantially α -helical in their soluble native folds have been found to form fibrils [4,5]. It has been recognised that in general the formation of fibrils occurs when the native protein is destabilised under conditions where noncovalent interactions, particularly hydrogen bonding remains favourable [3].

*Corresponding author: Dr. L.J. Smith, Central Chemistry Laboratory, University of Oxford, South Parks Road, Oxford, OX1 3QH, UK. Tel.: +44 1865 275961; Fax: +44 1865 275905; E-mail: lorna.smith@chem.ox.ac.uk.

In order to gain a full understanding into the process by which fibrils form it is necessary to characterise the properties of the conformers in solution from which aggregation and fibril formation occur. These conformers are often partially folded states and detailed structural studies of non-native proteins are challenging. Non-native states are in general ensembles of interconverting conformers [7]. Hence their study by NMR techniques is often hampered due to severe line broadening and lack of chemical shift dispersion [8]. One strategy therefore is to study the fibril formation of peptide fragments taken from a protein sequence since peptides can be more readily characterised at a sequence specific level using NMR techniques. This is the approach we use here. We report studies of fibril forming peptide fragments of differing lengths from the sequence of short consensus repeat 3 (SCR3) from the human complement receptor CR1 [9]. This receptor binds C3b and C4b, two complement proteins, and the peptides studied in this work include a region in SCR3/10 at which both proteins have been reported to bind. These interactions of CR1 with the complement proteins C3b and C4b form part of the catabolic and regulatory pathways of the immune system [10,11].

2. Materials and methods

Peptides SCR3(18–34) (sequence: STNRENFHYGSVVITYRS), SCR3(34–54) (sequence: SNPGSG-GRKVFELVGEPSIYS), SCR3(18–54) (sequence: STRENFHYGSVVITYRSNP GS GG RKVFELVGEPSIYS) and SCR3(27–33) (sequence: GSVVTYR) were synthesised using solid-phase methodology. The identity and purity of the final products were confirmed by FAB mass spectrometry and reversed-phase high performance liquid chromatography. The two cysteine residues (Cys 34 and Cys 54) within this region of the wild type protein sequence were replaced by serine residues. In addition in the SCR3(18–54) peptide Asn 20 has been deleted.

CD measurements were carried out on a Jasco J720 Spectropolarimeter at 25°C using a Haake F8 temperature controller. Far UV CD spectra were acquired using cells of 1 mm path length at 0.5 nm intervals over the wavelength range 190–250 nm. The concentrations of the peptides were determined by the UV absorption of the tyrosine residue at 274 nm. Peptide solutions were prepared by dissolving lyophilised peptide in deionised water (peptide concentration 50–100 μ M), and adjusting to pH 4 with small aliquots of NaOH and HCl. The H₂O/TFE cross titration experiments were carried out by mixing the appropriate aliquots of two 0.1 mM peptide stock solutions, one in water at pH 4.0 and the other in TFE. The peptide solutions in SDS were prepared by adding the peptide to a non-micellar 3 mM SDS solution in water at pH 4.

Samples for NMR spectroscopy were prepared to contain approximately 2 mM peptide at pH 4 in 10% D₂O/90% H₂O, 30% TFE-d₃/70% H₂O or 80% TFE-d₃/20% H₂O. ¹H NMR experiments were performed at 25°C using NMR spectrometers at the Oxford Centre for Molecular Sciences with ¹H operating frequencies of 500.2 and 600.2 MHz and an NMR spectrometer at the Center for Molecular and Structural Biology, LNLS with a ¹H operating frequency of 500 MHz. 2D experiments were acquired with 1K complex points in t_2 and in phase-sensitive mode using time-proportional phase incrementation (TPPI) for quadrature detection in t_1 . Typically 512–750 t_1 increments with 32–64 scans were recorded for TOCSY [12] (75 ms mixing time) and ROESY [13] (150 ms and 200 ms mixing times) experiments. For the determination of the ³J_{H_N α} coupling constants DQF-COSY spectra [14] were recorded with 1K t_1 increments of 4K complex data points. NMR data were processed using Felix 2.3 (Hare Research and Biosym Inc.) and Felix 2000 (Accelrys Inc.). The spectra were referenced to the internal standard DSS (2,2-dimethyl-2-silapentane 5-sulfonate) at 0.0 ppm. The spectra were assigned using standard

procedures [15] and the ^1H resonance assignments have been submitted to BioMagRes Bank (accession numbers 5731, 5732, 5733, 5734).

For the electron microscopy aliquots collected from the peptide solutions during fibril formation were applied to Formvar-coated grids. These were negatively stained with a solution of 2% (wt/vol) uranyl acetate in water, washed, air-dried and then examined in a JEOL JEM1010 transmission electron microscope operating at an accelerating voltage of 80 kV.

3. Results and discussion

In this work we concentrate on the sequence forming residues 18–54 of SCR3. Two peptides have been studied initially corresponding to residues 18–34 and 34–54 respectively (SCR3(18–34) and SCR3(34–54)). The structure of SCR3 has not yet been solved but the structures of a number of other short consensus repeat modules with a close homology to SCR3 are available [16] including the structure of the pair of modules SCR16–SCR17 from CR1 [17]. The sequences of SCR3 and SCR17 differ by only two residues in the region corresponding to the peptides studied. All these short consensus repeat modules have structures rich in β -sheet and no α -helical secondary structure [16]. Sequence alignment suggests that the peptide sequence SCR3(18–54) will form three β -strand segments in the native structure (residues 29–32, 46–47, 51–53) (Fig. 1).

3.1. Comparisons in aqueous and TFE solutions

As reported previously [18] incubation of SCR3(18–34) in aqueous solution at 25°C results in the formation of amyloid fibrils after a period of days. Under similar conditions SCR3(34–54) also forms fibrils (Fig. 2). To gain more insight into the properties of these peptides, and the conformations from which fibrils can form, CD and NMR studies have been performed for the two peptides in aqueous and TFE solutions.

Both the SCR3(18–34) and SCR3(34–54) peptides freshly dissolved in aqueous solution are essentially unfolded. The CD spectra (Fig. 3) show strong minima at *ca* 197 nm typical of a random coil and



Fig. 1. The structure of complement control protein 17 of CR1 [17] indicating in grey the residues corresponding to residues 18–34 of SCR3 and in black the residues corresponding to residues 35–54 of SCR3. The figure was generated using the program Molscript [34].

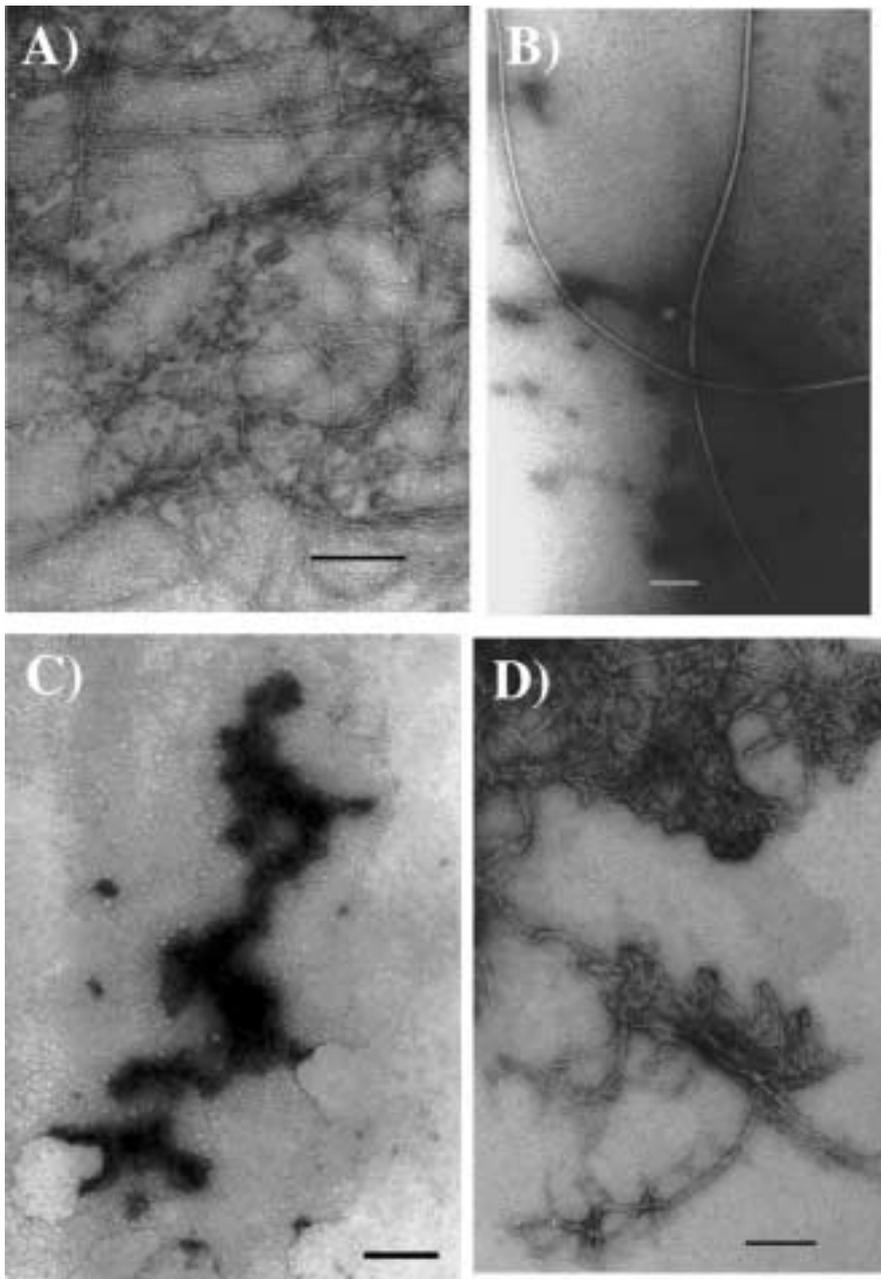


Fig. 2. Electron micrographs of fibrils formed by (A) SCR3(18–34), (B) SCR3(34–54), (C) SCR3(27–33) and (D) SCR3(18–54). In each case the fibrils were formed in 0.5 mM samples of the peptide in aqueous solution at pH 4 that had been incubated at room temperature for approximately 6 months (SCR3(18–34) and SCR3(34–54)), 3 months (SCR3(18–54)) or 1 month (SCR3(27–33)). Bar = 200 nm.

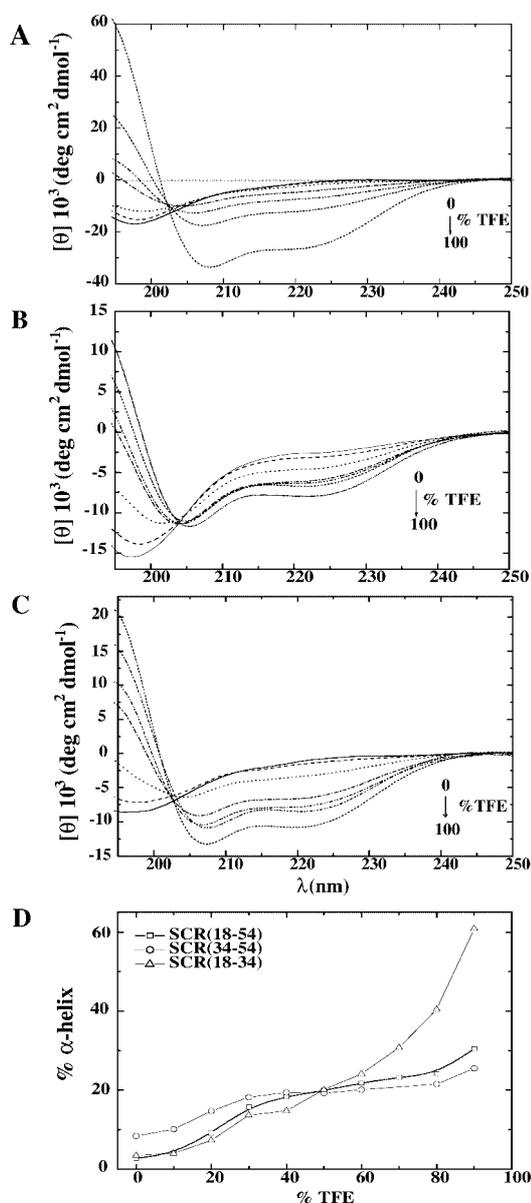


Fig. 3. Far-UV CD spectra of (A) SCR3(18–34), (B) SCR3(34–54) and (C) SCR3(18–54). In each case the bold line shows the spectrum of the peptide in aqueous solution and the dashed lines show the spectra for the peptide in increasing concentrations of TFE. Panel (D) shows the percentage of helicity present in each of the peptides in TFE solutions calculated from the ellipticity at 222 nm (symbols: SCR3(18–34) triangle; SCR3(34–54) circle; SCR3(18–54) square). In all the CD experiments a peptide concentration of 0.1 mM was used at pH 4 and 25°C.

the $C_{\alpha}H$ chemical shifts for the peptides (Fig. 4) are close to those seen for short unstructured GGXGG peptides [19]. Interestingly however a cluster of upfield chemical shift deviations (>0.1 ppm) are seen for Glu 22, Asn 23, Phe 24 and His 25 in SCR3(18–34). These deviations presumably reflect a persistent hydrophobic cluster involving the aromatic side chains of two of these residues, similar to those identified in a number of denatured proteins [20–22].

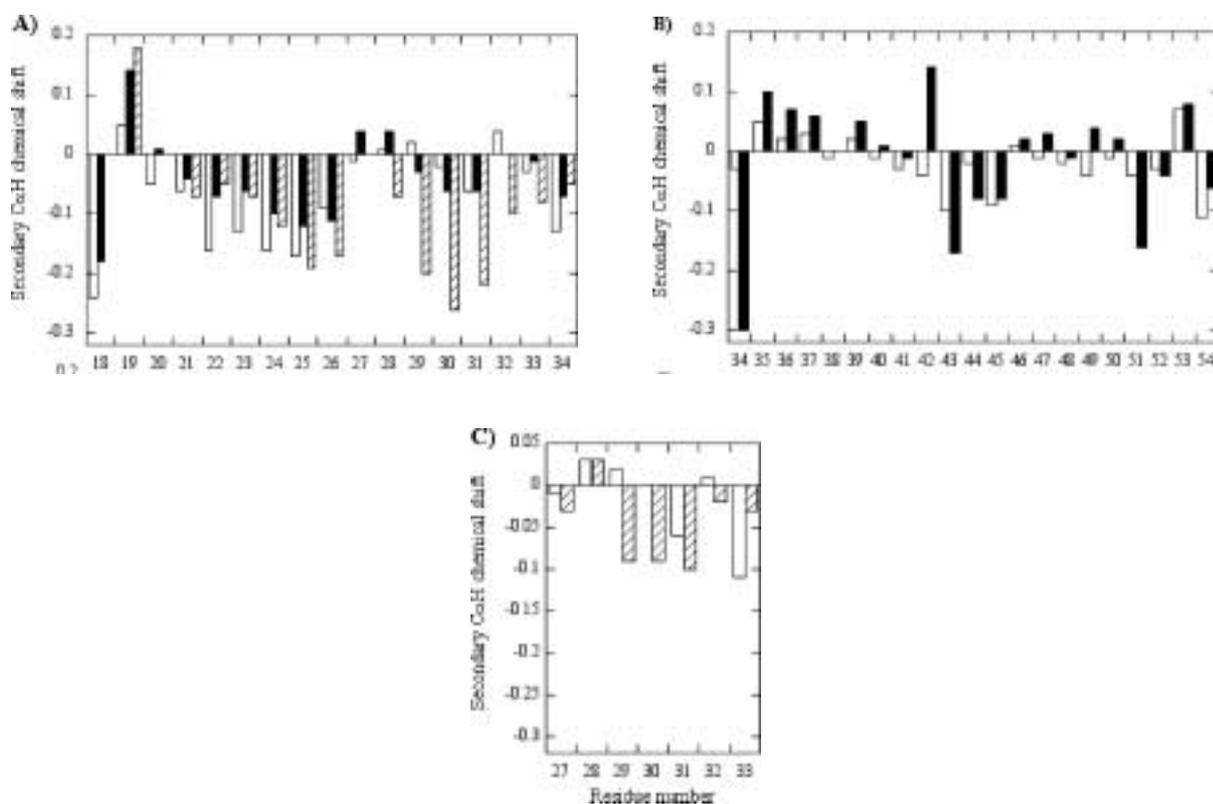


Fig. 4. Secondary $C\alpha H$ chemical shifts of (A) SCR3(18–34), (B) SCR3(34–54) and (C) SCR3(27–33). The open bars show the data for the peptides in aqueous solution, the filled bars the data for the peptides in 30% TFE and the dashed bars the data for the peptides in 80% TFE (pH 4 and 25°C). A positive secondary shift ($\Delta C\alpha H = \delta_{\text{measured}} - \delta_{\text{coil}}$) indicates a downfield chemical shift relative to the random coil values. The random coil chemical shifts of Merutka et al. [19] for GGXGG peptides have been used except for the residues that precede prolines. For these residues the values for GGXPGG peptides of Wishart et al. [35] have been used.

CD spectra were used to follow H_2O/TFE cross-titration experiments for the peptides (Fig. 3). In each case a transition to a more helical conformation, characterised by a negative band in the CD spectra near 210 nm and a shoulder at 220 nm, was observed on the addition of TFE. For SCR3(34–54) a sigmoidal increase in helical secondary structure was observed with increasing TFE concentrations indicating a cooperative induction of helicity. From the ellipticity at 222 nm SCR3(34–54) was estimated to contain 17.6% helicity in 30% TFE and 21.4% helicity in 80% TFE. The behaviour of SCR3(18–34) was different to that of SCR3(34–54). The peptide shows a regular increase in helicity throughout the titration having 13.6% helicity in 30% TFE and 37.3% helicity in 80% TFE. To gain further insight into these changes NMR studies of SCR3(34–54) in 30% TFE and SCR3(18–34) in 30% and 80% TFE were performed.

Comparisons of the $C\alpha H$ chemical shifts for SCR3(18–34) and SCR3(34–54) in 30% TFE with those seen for short unstructured GGXGG peptides [19] (Fig. 4) show that there are no clear pattern of chemical shift deviations for either peptide. For SCR3(18–34) deviations of more than 0.1 ppm are seen for the N-terminal residues Ser 18 and Thr 19 and for Phe 24–Tyr 26. The deviations for the latter residues presumably reflect the persistence of the hydrophobic cluster seen in aqueous solution. For SCR3(34–54) three residues have their resonances shifts upfield (Ser 34, Val 43, Ser 51) and one downfield (Lys 42)

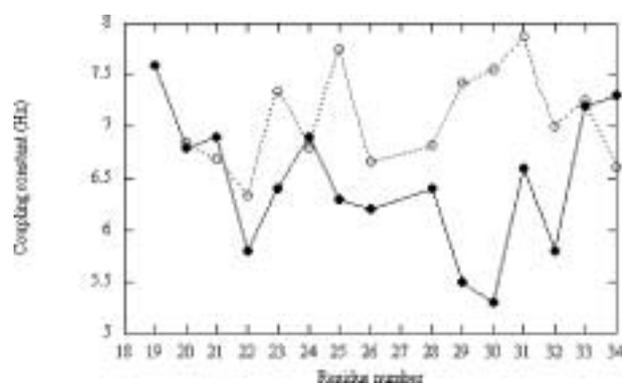


Fig. 5. $^3J_{\text{HN}\alpha}$ coupling constant values for SCR3(18–34) in aqueous solution (open symbols) and in 80% TFE (filled symbols) at pH 4. The coupling constants were measured at 35°C in aqueous solution and at 25°C in TFE.

by more than 0.1 ppm. Neither of the peptides shows any chemical shift deviations greater than 0.2 ppm (except for the N-terminal residue of SCR3(34–54)). The absence of large deviations presumably reflects the relatively small percentage helicity induced by 30% TFE (13.6% for SCR3(18–34) and 17.6% for SCR3(34–54)).

For SCR3(18–34) more significant upfield $\text{C}\alpha\text{H}$ chemical shift deviations (>0.2 ppm) are observed in 80% TFE for Val 29, Val 30 and Thr 31. As groups of upfield shifted $\text{C}\alpha\text{H}$ resonances are indicative of helical secondary structure [23] these data identify this 29–31 region as being the one that adopts helical conformers on the addition of TFE. Further evidence to support this comes from $^3J_{\text{HN}\alpha}$ coupling constant measurements (Fig. 5). In particular Val 29 and Val 30 have $^3J_{\text{HN}\alpha}$ values of 5.5 and 5.3 Hz respectively, values reduced by approximately 2 Hz from the coupling constants measured for these residues in the peptide in aqueous solution.

This helical structure adopted in TFE is particularly interesting as comparisons with the structure of SCR17 [17] suggest that residues 29–31 are a central β -strand (residues 29–32) within an antiparallel β -sheet in the native protein. Furthermore secondary structure prediction for SCR3 identifies the 29–31 sequence as having a very high β sheet propensity. For example, with the PHD method [24] residues 29–32 are predicted to adopt extended β -strand secondary structure with the maximum value (9) of the reliability index. Similarly using the Chou and Fasman method [25] the average conformational parameters for residues 29–32 ($\langle P_\alpha \rangle$ 0.88, $\langle P_\beta \rangle$ 1.36) show a strong preference for β -sheet secondary structure.

3.2. Comparison with the conformational properties of a longer peptide fragment

The β -sheet conformation adopted by at least some parts of the SCR3(18–34) and SCR3(34–54) sequences both when in the native folded protein and also when amyloid fibrils are formed presumably results from intra or intermolecular interactions that are absent when these short peptides are studied in a monomeric form. To investigate further the role of intramolecular interactions we have also characterised by CD and NMR techniques a longer peptide with a sequence corresponding to residues 18–54 of SCR3 (SCR3(18–54)). This peptide contains the residues that form the two central strands in the antiparallel β -sheet in SCR3. This SCR3(18–54) peptide has been found to form fibrils after a period of days of incubation in aqueous solution (Fig. 2D). However no fibril formation has been observed after incubation of the peptide in 80% TFE solution.

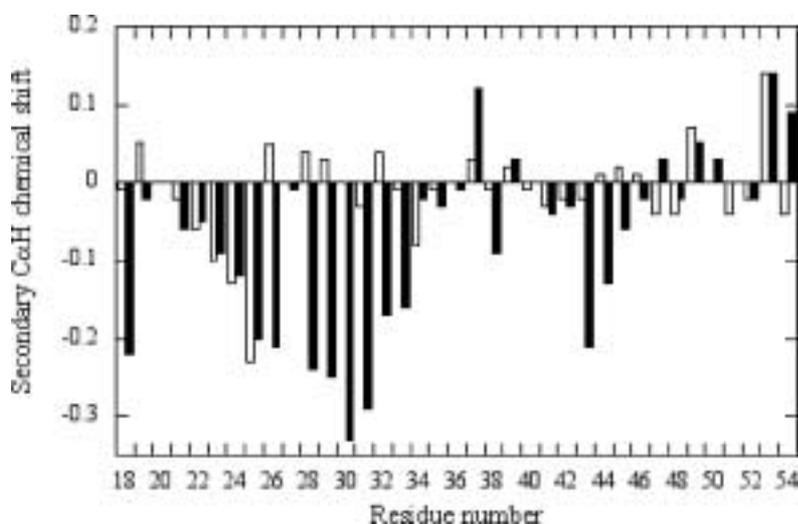


Fig. 6. Secondary C α H chemical shifts of SCR3(18–54). The open bars show the data for the peptides in aqueous solution and the filled bars the data for SCR3(18–34) in 80% TFE (pH 4 and 25°C). A positive secondary shift ($\Delta C\alpha H = \delta_{\text{measured}} - \delta_{\text{coil}}$) indicates a downfield chemical shift relative to the random coil values. The random coil chemical shifts of Merutka et al. [19] for GGXGG peptides have been used except for the residues that precede prolines. For these residues the values for GGXPGG peptides of Wishart et al. [35] have been used.

The CD spectrum the SCR(18–54) peptide freshly dissolved in aqueous solution is typical of a random coil and shows a strong minimum at *ca* 198 nm (Fig. 3C). The C α H chemical shifts for the peptides (Fig. 6) in aqueous solution are closely similar to those seen for the shorter SCR3(18–34) and SCR3(34–54) peptides. In addition the H₂O/TFE cross-titration experiment shows an increase in helicity on the addition of TFE similar to that observed for the shorter peptide fragments (Fig. 3C). Interestingly, however, in the longer SCR3(18–54) peptide in 80% TFE slightly larger C α H chemical shifts deviations are seen than for SCR3(18–34) under the same conditions. In particular Ser 18, His 25, Tyr 26, Ser 28, Val 29, Val 30, Thr 31 and also Val 43 all have deviations ≥ 0.2 ppm in SCR3(18–54) indicating that number of residues involved in the helicity is increased in the longer peptide.

Despite the longer peptide sequence of SCR3(18–54), there is no evidence for a significant population of β strand in the SCR3(18–54) peptide in aqueous solution or TFE. We have therefore also studied the peptide in sodium dodecyl sulfate (SDS), a detergent that is known in some cases to promote β -sheet conformers. Under these conditions the CD spectrum of SCR3(18–54) is characteristic of a β -strand conformation. In particular in 3 mM SDS below the critical micellar concentration a strong band just below 210 nm and a negative band at 220 nm are observed. This behaviour for SCR3(18–54) differs to that of SCR3(18–34) in SDS. Here, as we have reported previously, the peptide initially acquires an α -helical conformation in the presence of non-micellar concentrations of SDS. However under these conditions the SCR3(18–34) peptide then converts to a β -sheet and large quantities of fibrils form [18].

3.3. A short peptide fragment will also form fibrils

In SDS both the SCR3(18–54) and SCR3(18–34) peptides are observed, under certain conditions, to adopt β conformers. However under the conditions of the NMR studies of the peptides in aqueous solution and TFE there was no evidence for β conformers. Therefore to obtain more information on a residue specific level as to which residues in the 18–54 sequence may be important in the fibril formation

we have also characterised a shorter peptide fragment taken from this sequence of SCR3. The peptide corresponds to residues 27–33 (SCR3(27–33)), one of the regions that comparisons with the structure of SCR17 suggests contains a β -strand in the native folded structure. Reflecting this, residues in the centre of the peptide sequence are predicted to adopt β -strand secondary structure with the maximum reliability index (with the PHD method [24]). However the peptide also contains the group of four residues which in the SCR3(18–54) peptide in TFE have upfield chemical shift deviations ≥ 0.2 ppm characteristic of α -helical conformations (for residues 28–31).

SCR3(27–33) was found to be essentially unfolded in aqueous solution, the CD spectrum resembling that expected for a random coil. On incubation the peptide in aqueous solution was found to form fibrils (Fig. 2C) after a period of days. The CD spectrum of SCR3(27–33) showed evidence for an increase in helicity on the addition of TFE but NMR studies indicate that the helical secondary structure is less persistent than in the longer peptides. In particular in 80% TFE the only $C\alpha H$ chemical shift deviation ≥ 0.1 ppm is for Thr 31 (-0.10 ppm) (Fig. 4C). This presumably reflects the fact that the short peptide length does not give possibilities for many helix-stabilising hydrogen bonding interactions. However under these conditions in 80% TFE SCR3(27–33) was not found to form fibrils.

4. Conclusions

The 18–54 sequence from SCR3 has a clear propensity to form fibrils. Indeed all the peptides reported here have been found to form fibrils when in aqueous solution including even a short peptide of just seven residues (SCR3(27–33)). In aqueous solution the peptides are essentially unfolded and therefore, as in a model random coil, each residue will be interconverting between α and β main chain conformations [26]. The sequence, however, has a β propensity and the native SCR3 fold is rich in β -sheet. Amyloid fibrils have also been shown to contain extensive β structure [27]. Therefore for the isolated peptide fragments aggregation and formation of fibrils lead to the stabilisation of preferred β conformers in the absence of the tertiary interactions that would be present in the full protein structure. The results for these SCR3 peptides therefore support hypothesis that the ability to adopt amyloid fibrils is an inherent property of the polypeptide backbone [6].

In TFE solutions studies of both SCR3(18–54) and SCR3(27–31) have not given any evidence of fibril formation. CD studies show that the peptides have an increased helicity under these conditions. For most parts of the sequence however the NMR chemical shift deviations for the peptides in TFE from the values expected of a random coil are relatively small. This indicates that the helical secondary structure adopted by the peptides in TFE must be fluctuating and dynamic in nature. Studies of acylphosphatase have shown that for this protein fibril formation is accelerated in 25% TFE. However at higher concentrations of TFE ($>35\%$) fibril formation does not occur [28]. For acylphosphatase it is proposed that at the higher TFE concentrations aggregation is not favoured because of increased intramolecular hydrogen bonding and reduced hydrophobic effects [28]. These factors are presumably also responsible for the inhibition of fibril formation for this sequence in TFE. As any intramolecular hydrogen bonds will be very fluctuating for the peptides it is likely that the reduced hydrophobic effects may be the dominant factor. Indeed the SCR3 18–54 sequence has a high hydrophobicity containing 12 residues with aromatic side chains or other significantly hydrophobic side chains (Val, Leu, Ile). In addition in aqueous solution residues 22–25 have been identified to form a hydrophobic cluster.

It is interesting that the part of the SCR3 sequence studied which the NMR studies shows has the highest α -helicity in TFE (residues 28–31) has a high β -sheet preference and a significant propensity to

form fibrils. A number of other studies have identified sequences that adopt β -strands in native protein folds but α -helices in TFE including parts of β -lactoglobulin and hen lysozyme [29–31]. In the cases of these proteins though, in contrast to the SCR3 residues, the sequences have a high α -helical propensity. This local helical preference dominates in the absence of longer-range intramolecular contacts. Some understanding into the helicity observed in SCR3 despite the high β -sheet propensity comes from the results of studies of the changes in the conformational propensities of different amino acids in TFE [32, 33]. These have shown that increases in helical preference under these conditions are context dependent but there is a significant increase in the helical propensity of amino acids with nonpolar β -branched side chains such as valine. As the 28–31 sequence contains two valines (sequence SVVT) it is therefore presumably these residues that are at least in part responsible for the helicity in TFE. Interestingly though these same valine residues are also likely to be important in determining the β -sheet preference in the native protein and the propensity to form fibrils. The variety of conformations observed for this sequence therefore provide a clear example of the manner in which the environment can significantly influence conformational preferences of a polypeptide chain.

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