

Stimulation and inhibition of fibril formation by a peptide in the presence of different concentrations of SDS

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Abstract Sodium dodecyl sulphate (SDS), a detergent that mimics some characteristics of biological membranes, has been found to affect significantly fibril formation by a peptide from human complement receptor 1. In aqueous solution the peptide is unfolded but slowly aggregates to form fibrils. In sub-micellar concentrations of SDS the peptide is initially α -helical but converts rapidly to a β -sheet structure and large quantities of fibrils form. In SDS above the critical micellar concentration the peptide adopts a stable α -helical structure and no fibrils are observed. These findings demonstrate the sensitivity of fibril formation to solution conditions and suggest a possible role for membrane components in amyloid fibril formation in living systems.

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1. Introduction

Amyloid fibril formation is associated with a wide range of human debilitating disorders such as Alzheimer's and Creutzfeldt–Jakob diseases, senile systemic amyloidosis and type II diabetes [1–5]. Initially it was generally believed that only a small group of proteins could form the highly ordered fibrillar aggregates associated with these diseases. It has recently been shown, however, that the ability to form fibrils *in vitro* is not limited to the 20 or so disease-associated proteins [5–10]. Indeed, it has been suggested that the ability to form this type of structure may be intrinsic to the polypeptide backbone, and a generic property of polypeptide chains, although the propensities of different sequences to form such species can differ widely [7,9].

The conversion of globular proteins into fibrils usually involves major structural transitions leading to the partial or complete disruption of the native fold under conditions in

which non-covalent interactions, such as hydrogen bonding, remain favourable [7]. Aggregation of this type, therefore, generally occurs under partially denaturing conditions or on fragmentation of the polypeptide chain into segments that cannot form the cooperative native structure of the protein. α -Helical to β -sheet transitions play an essential role in the process of fibril formation if the native form of the protein is largely helical, as for example in the case of the prion protein, as the aggregated amyloid structures are rich in β -sheet [11,12]. Indeed it has been shown that stabilising α -helical conformers in a denatured or non-globular ensemble relative to more extended β -structures can reduce substantially the propensity for aggregation and amyloid formation [13,14].

In order to explore the role of α to β transitions in fibril formation in more detail we have examined the effects of the detergent sodium dodecyl sulphate (SDS) on the solution conformation and the propensity to form fibrils of a peptide. SDS above its critical micellar concentration (cmc) is known to stabilise peptides in helical conformations [15,16], while below its cmc SDS has been found to stabilise β -strands [17,18]. It therefore provides an opportunity to alter the relative stability of different conformations of a peptide simply by changing the SDS concentration. Moreover, SDS mimics some properties of lipid membranes and enables us to speculate on the possible role of the lipid components of membranes or the membrane environment on the potential to form amyloid fibrils.

The peptide studied in the present work is SCR3(18–34), a 17 residue fragment corresponding to residues 18–34 of the SRC3 (short consensus repeat 3) domain from the human complement receptor CR1 [19]. This receptor binds two active complement proteins, C3b and C4b, and these interactions form part of the catabolic and regulatory pathways of the immune system. The 18–34 sequence spans a region in SCR3/10 of CR1 at which both proteins have been reported to bind [20]. The peptide was chosen for the present study because it was found to form spontaneously very well-defined amyloid fibrils under conditions that are readily studied experimentally.

2. Materials and methods

The peptide used here (STNRENFHYGSVVITYRS) was synthesised by solid-phase methodology. The identity and purity of the final product was confirmed by mass spectrometry (experimental mass 2016.1, calculated mass 2014.9) and reversed-phase high performance liquid chromatography. SDS was purchased from Sigma (St. Louis, MO, USA).

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Abbreviations: CD, circular dichroism; cmc, critical micellar concentration; EM, electron microscopy; SDS, sodium dodecyl sulphate

Circular dichroism (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 a cell of 1 mm path length at 0.5 nm intervals over the wavelength range 190–250 nm. The concentration of the peptide was determined by the UV absorption at 274 nm of the single tyrosine residue ($\epsilon_{274} = 1420 \text{ M}^{-1} \text{ cm}^{-1}$). Solutions of the peptide were prepared by dissolving lyophilised peptide in deionised water, and the pH adjusted to 4.0 with small aliquots of NaOH and HCl, or by adding the lyophilised peptide to solutions of SDS in water at pH 4. The final concentration of peptide in aqueous solution was set to 50 μM and the SDS concentrations to 3 mM or 20 mM.

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

3. Results

3.1. Formation of fibrils by SCR3(18–34) in aqueous solution

SCR3(18–34) adopts a predominantly random coil conformation when initially dissolved in aqueous solution at pH 4 and 25°C. This is illustrated by the CD spectrum of the peptide in Fig. 1A in which a single minimum can be observed at 198 nm. A shoulder at 215 nm and a band at 227 nm are, however, also observed in the spectrum and can be attributed to the presence of a small proportion of turns or more extended regions of β -sheet structure in the conformational ensemble. After two days of incubation at room temperature, however, significant changes were observed in the intensity of the CD spectrum (Fig. 1A). The spectrum is still characteristic of a peptide in the random coil conformation, with a single minimum at ca. 195 nm, suggesting that no significant structural changes have occurred in the peptide giving rise to the CD spectrum. However, over that time period, the intensity of the bands in the spectrum has decreased by a factor of at least two. Electron micrographs of the sample reveal the presence of a small number of well-defined fibrils (Fig. 1B). The fibrils detected are unbranched, straight or slightly curved, and have diameters of 5–10 nm. They are of variable length and show no twisting pattern along the fibre axis. The decrease in intensity of the CD spectrum observed after 2 days of incubation can therefore be associated with the formation of fibrillar species too large to give rise to significant CD absorption.

Fig. 1C shows electron micrographs of the sample taken after a very prolonged period (24 weeks) of incubation at pH 4 and room temperature. This micrograph reveals the emergence of a dense network of fibrils. The fibrils are long, unbranched and have a diameter of ca. 5–10 nm. Although the sample shows a high degree of homogeneity in the morphologies of the fibrils some variations can be observed in their appearance. In particular, two types of assemblies are evident. The most common fibrils have a smooth non-twisted appearance, similar to those observed after just 2 days of incubation while the others are twisted but with no well-defined helical periodicity. These fibrils appear to be the result of the association of two or more distinct strands or protofilaments, a situation commonly observed in mature amyloid fibrils [21–23]. The CD spectrum corresponding to the 24 week sample of SCR3(18–34) is also shown in Fig. 1A and reveals a dominance of bands characteristic of β -sheet structure with a minimum at 216 nm and a positive band at ca. 237 nm. This result suggests that conversion of soluble SCR3(18–

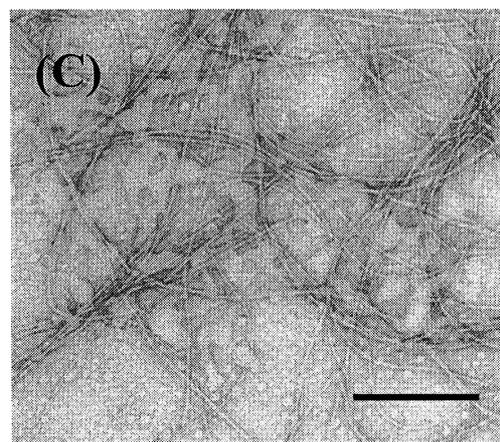
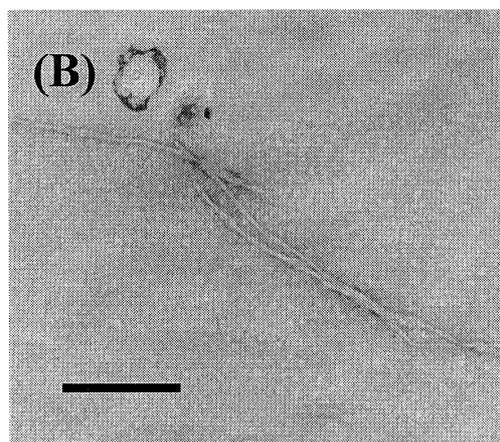
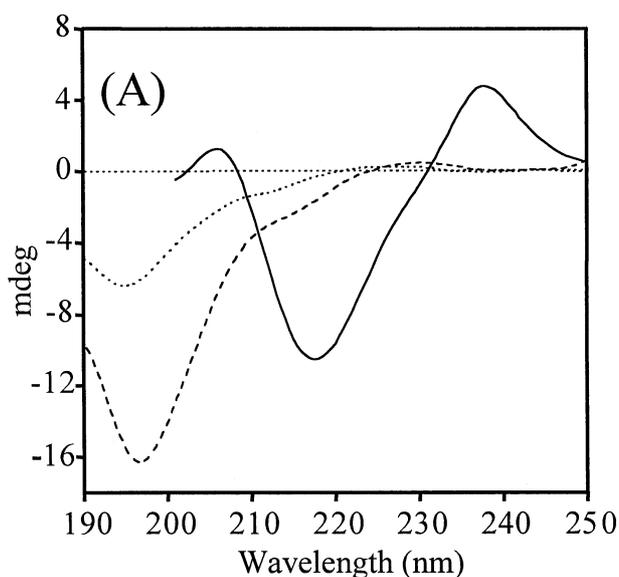


Fig. 1. CD spectra (A) of a 50 μM solution of the SCR3(18–34) peptide freshly dissolved in aqueous solution at pH 4 and room temperature (dashed line), and after 2 days (dotted line), and 24 weeks (solid line) of incubation at room temperature. Electron micrographs SCR3(18–34) fibrils after 2 days (B) and 24 weeks (C) of incubation at room temperature. Bars = 200 nm.

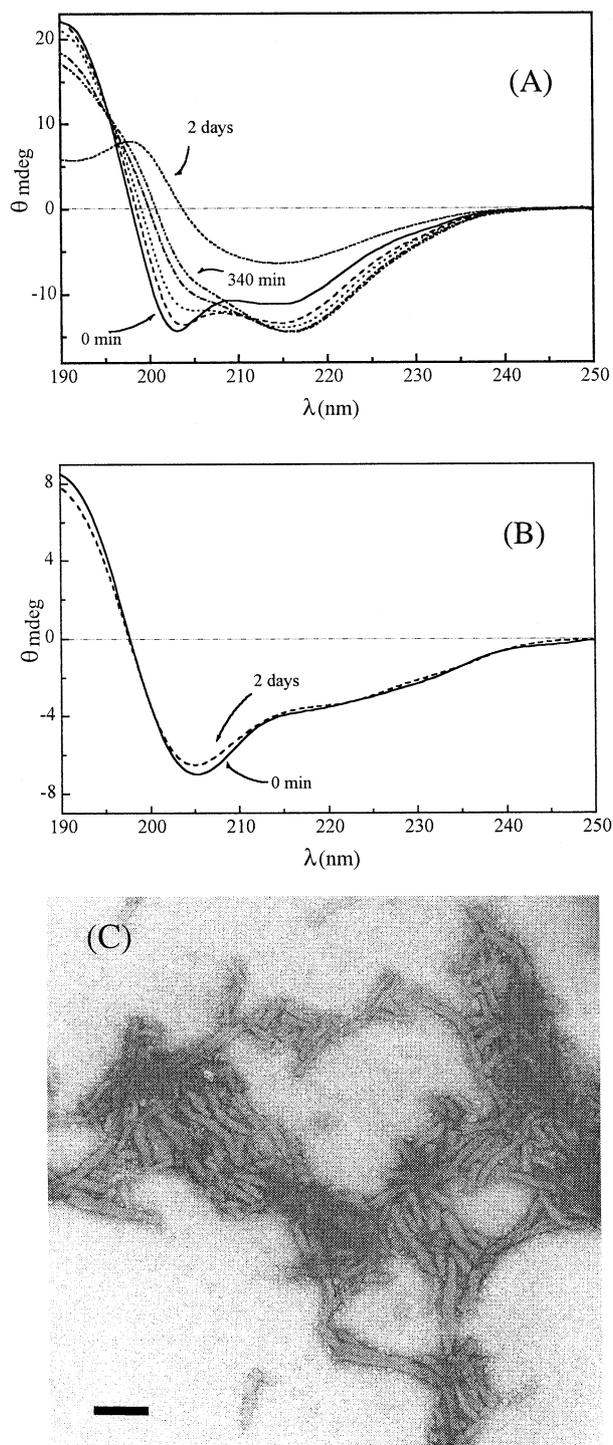


Fig. 2. A: Time evolution of the CD spectra of a 50 μM SCR3(18–34) peptide solution in the presence of 3 mM SDS at pH 4 and 25°C: 0 min (solid line); 30 min (dashed line), 70 min (dotted line), 180 min (dot-dashed line), 340 min (dot-dot-dashed line) and 2 days (dashed line). B: CD spectra of SCR3(18–34) in the presence of 20 mM SDS at pH 4 and 25°C freshly dissolved (solid line) and after 2 days (dashed line) of incubation at room temperature. C: Electron micrograph of 50 μM of SCR3(18–34) in the presence of 3 mM SDS at pH 4, after 2 days of incubation at room temperature. Bar = 200 nm.

34) to amyloid fibrils is accompanied by a transition from a random coil to β-sheet structure and that the fibrils observed are rich in β-sheet structure.

3.2. SDS as a promoter of fibril formation

CD spectra of SCR3(18–34) recorded in the presence of a sub-micellar (3 mM) concentration of SDS are shown in Fig. 2A. The spectrum of the peptide recorded immediately after dissolution in SDS is dramatically different from that observed for the peptide in aqueous solution alone. The spectra show a positive peak at 190 nm and two minima located at ca. 203 nm and 216 nm. The positions and their relative intensities suggest that the peptide has acquired a substantial degree of helical structure. The positions of the peaks are, however, shifted from those typical of α-helical conformations by ~5 nm; it is possible that this shift is indicative of distortions to the regular helical structure, but could also be the result of light scattering from some aggregated material [24].

The CD spectrum recorded after 30 min is, however, significantly changed from that described above; the peak at 203 nm is of lower intensity and there is an increased intensity of the spectral component at 216 nm. The CD spectra collected over 2 days show a transition to a curve typical of β-sheet structure having a single minimum at 216 nm. The spectra recorded for the first 340 min show a well-defined isodichroic point at 195.5 nm suggesting that the helical to sheet transition can, to a reasonable approximation, be described as a two-state process. The species giving rise to the signals characteristic of β-sheet structure are likely to be due to the presence of small aggregates because the formation of large aggregates, as discussed above, leads to a loss of spectral intensity from scattering effects. The samples of SCR3(18–34) incubated in the presence of sub-micellar SDS were also analysed by EM (Fig. 2C). The results reveal the formation of a large quantity of fibrillar structures. Relative to those formed in the absence of SDS, the fibrils are very short but have a much larger diameter (13–32 nm).

Fig. 2B shows the CD spectrum of SCR3(18–34) in the presence of a higher concentration (20 mM) of SDS such that it forms micelles. The spectrum is highly characteristic of an α-helical conformation, with two minima at ca. 205 nm and 222 nm and a positive band at ca. 190 nm. The spectrum at room temperature was found to remain effectively unchanged over periods of time in excess of 2 days. Under these conditions, therefore, the peptide appears to form a stable α-helical structure that does not convert into amyloid fibrils over the time scale investigated here.

4. Discussion

The results described in this paper show that SCR3(18–34) can be added to the increasingly long list of peptide and proteins that have been shown to form well-defined fibrillar aggregates under particular solution conditions. It adds to the evidence that the ability to form amyloid fibrils is a common or generic property of polypeptide chains, dominated by the interactions of the main chain that is common to all sequences [5]. The CD data indicate that in aqueous solution the peptide adopts a conformation without discernible non-random structure. The aggregation process under the conditions explored here is very slow, but leads to the formation of a large number of long, well-defined fibrils. The structure of the fibrils is typ-

ical of those produced by other peptides as a result of slow growth in the absence of denaturant or co-solvent [21–23]. In these cases, it has been suggested that the observed twisting of the fibrils is a consequence of the greater overall stability resulting from factors such as the burial of exposed hydrophobic surfaces and an increase in van der Waals interactions [22,25].

In the presence of sub-micellar concentrations of the detergent SDS the peptide initially adopts a well-defined helical structure. After a short time, however, the CD spectrum changes to one characteristic of β -structure and a large number of fibrillar species are visible in the electron micrographs. These fibrils are much shorter and thicker than those formed by slow growth in aqueous solution. The development of β -structure over time is highly indicative of the formation of aggregates and has frequently been observed for peptide fragments [26]. The altered appearance of the SCR3(18–34) fibrils in SDS relative to those formed in pure water may be a result either of the rapid growth of the fibrils or a result of the presence of SDS. It is well established that the morphology of the assembly of amyloid protofilaments into mature fibrils is highly dependent on solution conditions, primarily because these conditions are likely to modulate the relatively weak interactions between the highly stable component protofilaments [25]. It is also possible that SDS is incorporated in some way into the structures, perhaps binding to the protofilaments prior to their assembly.

Low concentrations of SDS promote rapid fibril formation despite the fact that the initially formed, presumably monomeric, species are helical. A similar conversion of a soluble α -helical non-globular state to β -rich aggregates has previously been observed for the protein acylphosphatase in moderate concentrations of TFE, although at high concentrations aggregation is strongly inhibited [7]. In the presence of TFE, fibril formation may be stimulated not just by unfolding but by an enhanced stability of hydrogen bonding interactions. In the case of SDS, it is likely that the alkyl chains of SDS stimulate aggregation, perhaps by association with the peptide molecules. Once such aggregates are formed, intermolecular β -sheet interactions are likely to develop readily. Moreover, it has been shown that even proteins such as myoglobin and cytochrome *c*₅₅₂, that have sequences with high propensities for helical structure can form amyloid fibrils under appropriate conditions [9,10]. It is clear, however, that promotion of helical structure in the absence of any other factors is likely to inhibit aggregation by increasing the population of species that form intramolecular hydrogen bonds, and hence reducing the propensity to form intermolecular interactions [13,14]. In this context it is interesting that in the presence of higher concentrations of SDS, in which micelles are formed, fibril formation is not observed for the SCR3(18–34) peptide. Under these conditions the peptide again forms a helical structure initially but the helical structure still remains over long periods of time.

The ability of SDS both to promote fibril formation and to inhibit it, depending on conditions, is particularly interesting in the light of suggestions that interactions with membranes and membrane components may be significant in influencing aggregation and amyloid fibril formation in vivo [27]. For example, interactions between the amyloid β -peptide and lipid molecules have been suggested as playing an important role in the pathogenesis of Alzheimer's disease [28]. In addition, a

partially folded form of human α -lactalbumin interacting with a lipid cofactor has been found to induce apoptosis in tumour cells suggesting that the toxicity of non-native protein species can be dramatically influenced by lipid binding [29]. Overall the finding in this work that SDS can both stimulate and inhibit fibril formation reflects the sensitivity of this process to the solution environment, a factor that is undoubtedly a major aspect of the deposition and hence the toxicity of fibrils in biological systems [30].

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