Fast gelation of self-assembling peptide nanofibres triggered by electromagnetic radiation heating

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ABSTRACT

Peptides and peptide amphiphiles are able to form nanoscale ordered hydrogels. We report a method for the preparation of such nanofibre based gels. These gels are prepared by a rapid microwave heating and cooling cycle.

An amphiphilic derivative of the hydrophilic hexapeptide dalargin, in which a C_{16} acyl chain is attached via an ester linkage to the tyrosine residue (palmitoyl dalargin – pDal) was used to construct the nanofibre gels. The thermal properties of pDal were analyzed using differential scanning calorimetry (DSC) and Hot Stage Microscopy (HSM). When a dilute dispersion of pDal freeze dried solid (1mg mL^{-1}) is microwave heated in short bursts, at the acyl chain melting temperature (88°C, 10) nanofibres with a diameter of 8-20 nm are formed; heating a more concentrated dispersion (10 mg mL^{-1}) results in the formation of a nanofibre gel. Our findings show that microwave heating of peptide amphiphiles, bearing acyl chains is a reproducible method of producing peptide nanofibre gels.

Keywords: peptide, self-assembly, nanofibres, gel, microwave.

1 INTRODUCTION

Peptide nanofibrous systems mimic the architecture of naturally occurring cellular structures (i.e tethers, extracellular matrix components), present a high surface to volume ratio, and are constituted of biocompatible materials. For these reasons they represent a good candidate for applications in the field of regenerative medicine, cell signaling carriers and drug delivery systems. Naturally occurring and synthetic peptide amphiphiles self-assemble into high axial ratio fibrillar nanostructures. On prolonged exposure to high temperature, these nanofibre dispersions further self assemble into bundles and plaques on cooling [1].

Here we report a fast and facile method for the preparation of gels based on peptide self-assembling nanofibres. These gels are prepared by a rapid microwave heating and cooling cycle. These materials can provide new transporting biocompatible materials that form efficient networks of nanochannels with potential application, for instance, as a depot system for sustained release of pharmaceuticals.

2 RESULTS AND DISCUSSION

Self-assembly is a naturally occurring event, however the kinetics of the nanofibrils formation mean it can take days to get to completion [2]. Nanofibres made of surfactant-like peptides have been prepared with probe sonication and microwave heating [3]. Sonication of proteins is already known to cause formation of fibrillar aggregates [4], but to the best of our knowledge this is the first report of fibrillar gel formation using microwave heating. High local temperatures generated by sonication or by microwave bursts appear to promote single peptide molecule unfolding and enhance the aggregation of the single monomer units into nanofibres by promoting the hydrophobic interaction of the sixteen carbon chains and the formation of hydrogen bonds in the β-sheet structure among the peptide sequences. It has been observed that α-helical proteins such as Bovine Serum Albumin undergo conversion to β-sheet structure upon sonication [4]. We have prepared a self-assembling peptide nanofibre gel by applying a rapid microwave heating and cooling cycle to a concentrated dispersion of pDal (10 mg mL^{-1}, Figure 1A and B)

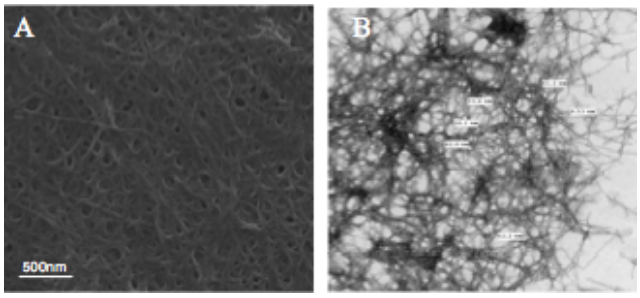

Figure 1: SEM pDal xerogel with a 5nm gold coating (A); TEM of pDal gel stained with uranyl acetate (B).

pDal was synthesised using solid-phase peptide synthesis and fluorenylmethoxycarbonyl based chemistry. The product was freeze dryed to obtain a white fluffy powder

[3]. Thermal analysis of pDal helps to clarify the thermal properties of the monomeric starting material and the influence of these properties on the molecular self-assembly. Thermal analysis also clarify the range of temperatures to be used for the preparation of these gels that have potential applications as drug delivery system.

Thermal stability of fibrillar collagen like structure of peptide amphiphiles was found to increase as the monoalkyl tail chain length is increased over a range of C_6 to C_{16} in studies with circular dichroism [5], thus increasing temperature to form the gels will not cause degradation of the peptide molecules.

The freeze dried pDal has amorphous characteristics, such as the lack of three-dimensional order characterized by the absence of sharp peaks in powder XRD (Figure 2), a glass transition (Figure 3A) and the formation of a glassy solid material upon heating (Figure 4E-H).

DSC analyses (Figure 3A) of the freeze-dried peptide showed tree endothermic events, at low temperature a broad peak (peak I), as well as two high temperature endothermic transitions: one sharp peak with onset at 85°C (peak II) which merges with a broader endothermic peak (peak III). Peak I is due to solvent loss, probably entrapped during the freeze drying process (indeed cooling down the system and heating up again shows that the event is not recoverable-data not shown). Peak (II), with an onset of 85°C (typical of hydrocarbon chain melting [6]), is followed by a broad endotherm. Althoug not conclusively proven, peak III could be a liquid crystal transition [7], which is in conflict with the definition of the sample being amorphous. We will return to this apparent dichotomy in the discussion. Upon DSC analysis, the sample melts and becomes a glassy solid upon cooling to room temperature. The glassy solid, when subjected to a second DSC heating cycle under the same conditions, showed a step in the baseline typical of glass transition (T_g), confirming the material formed was a glass.

Peak III is a non-thermoreversible process as upon cooling of the sample the transition was not recovered (Figure 3B). Annealing the glass formed after the first DSC cycle at a temperature below the T_g for up to 2 weeks showed recovery of the endothermic peak of the alkyl chain melting as well as enthalpy relaxation at the T_g (Figure 3B).

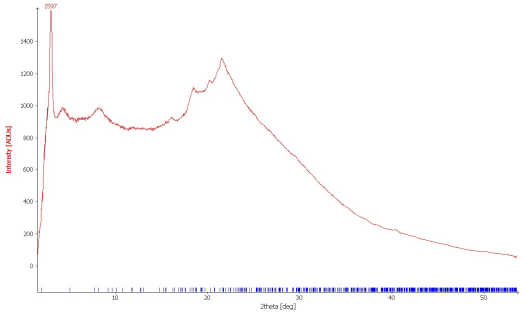

Figure 2: X-ray powder diffration for freeze dried pDal

Glasses that have undergone enthalpy relaxation will exhibit enthalpy recovery during heating, which is manifested as an overshoot in the heat capacity near Tg.

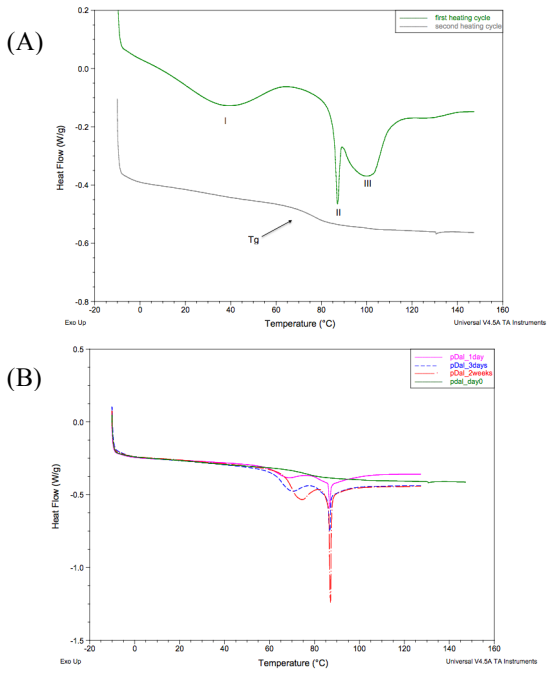

Figure 3: (A) DSC scans of pDal freeze-dried; (B) DSC scans of aged glassy solid. Heating rate 10°C/minute

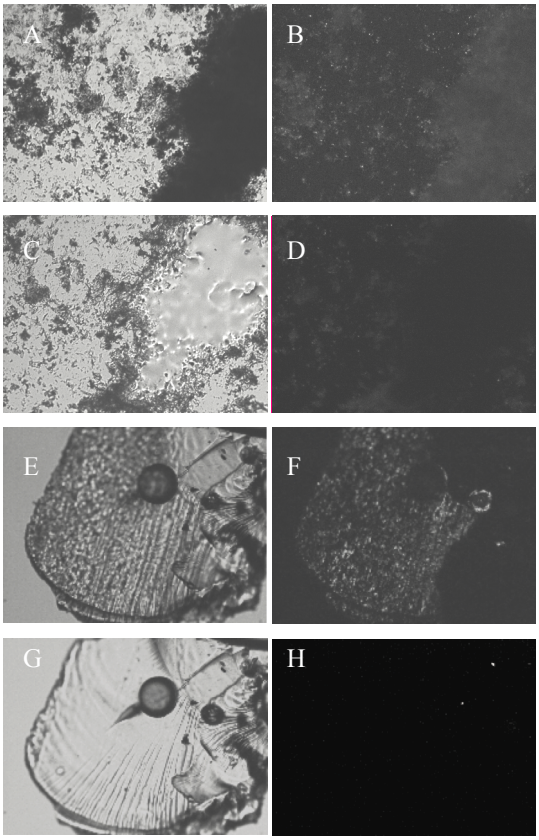

Figure 4: Optical micrograph of pDal:(A) powder at 25 ^oC;(B) under cross-polarized filters;(C) powder at 93^oC(D) under cross-polarized filters;(E) aged glass at25 ^oC,(F) under cross-polarized filters; (G) aged glass at 93^oC, (H)under cross-polarized filters.

Microscopic examination of the freeze-dried powder under cross-polarized filters showed some birefringence, which would not have been expected if the material was a true amorphous glass (Figure 4A-B). A phase transition was observed when the material reached 93°C whereupon birefringence was lost (Figure 4C-D). A similar observation was noted when the aged glass was studied under the microscope (Figure 4E-H).

Microwave experiments have highlighted that heating the solution up to the Tg temperature did not result in the formation of gels; increasing the heat generated from the electromagnetic radiation was necessary to obtain a gel upon cooling of the solution. The heated sample was left to cool unperturbed at room temperature for at least 30 minutes; alternatively the sample was directly placed in the fridge (5°C) for 10minutes to recover the gel (Figure 5C).

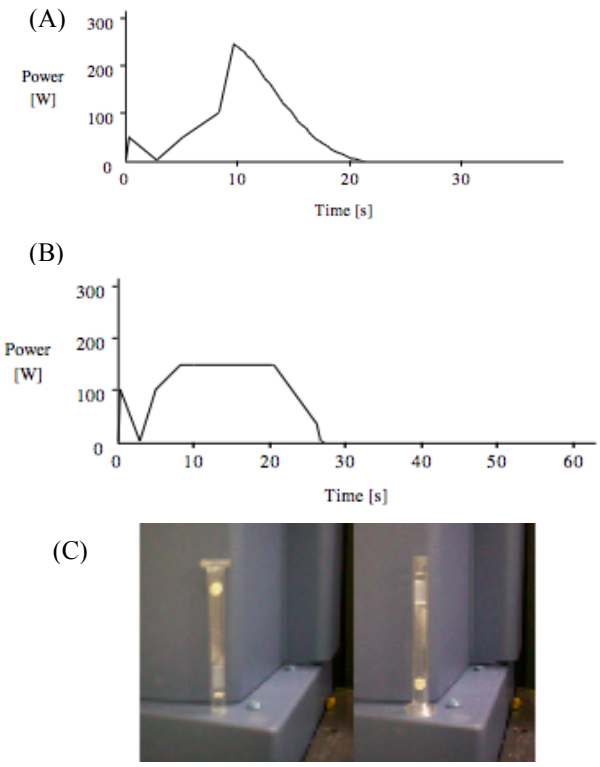

Figure 5: Power profiles for the microwave experiments, (A)when the experimental temperature = T_g; (B) when the experimental temperature = melting temperature; (C) inverting the sample vial upon cooling shows that the gel does not flow.

Microwave radiation causes the surfactant-like molecules to melt, creating two partially miscible fluids (as the peptidic portion of the molecule is water soluble). However the conformational disorder of the lipid chain due to the water-palmitic tail moiety contact is very high and so the system needs to escape it. We can thus hypothesize that the system rearranges in a a three dimensional ordered array with bicontinuous micellar cubic phase. For example, lamellar phases formed by biological amphiphiles (i.e. membrane bilayer, one of the ubiquitous lyotropic liquid crystal forms), can escape curvature frustration arising from an increase of temperature by becoming an inverse hexagonal phase formed of inverted cylindrical micelles packed into an hexagonal lattice [8]. However, because of the presence of voids in the packing that are available to solvating molecules, a "packing frustration" still exist due to these hydrophobic volume areas that the system cannot tolerate and some chains must deviate from their preferred conformational state in order to eliminate the hydrophobic voids [8]. Thus, as for lyotropic liquid crystals, the palmitic chains may rearrange in a 3D ordered array of lamellar bicontinuous micellar cubic phase such that the water-chain contact is minimized determining the formation of a nanofibre gel with nanoporous size.

When the sample is heated above its T_g or melting temperature there is a concomitant increase in the power supplied by the microwave in order to attain the programmed heating rate (Figure 5B). This is a reflection of the incrreased molecular mobility (effectively heat capacity) in the sample and is the same phenomenon that underpins analysis by DSC.

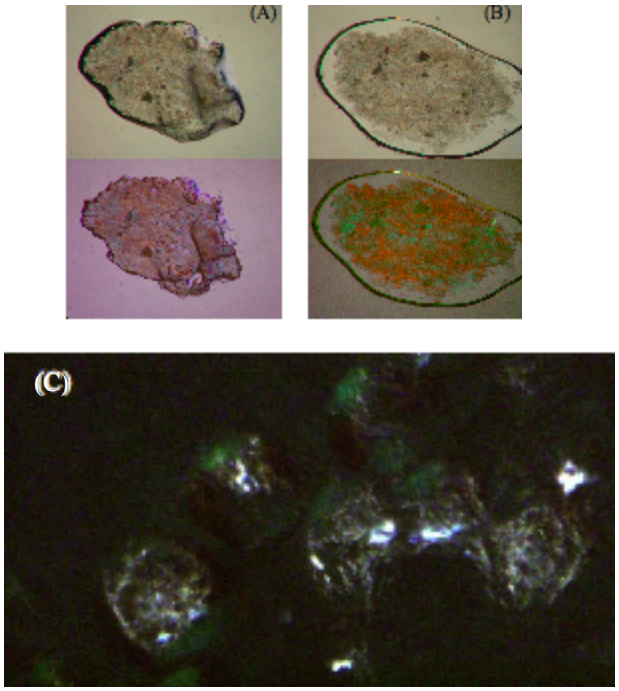

Figure 6: pDal gel (A) and sheared gel (B)under polarising light micorscope; (C) pDal gel stained with Congo Red.

The pDal gel sheared between a microscope slide and a cover slide displayed a color pattern when seen under polarized light (however XRD of the gel showed no sharp diffraction peaks – data not shown). In order to elucidate the nature of the interactions between the molecules in the tight entangled gel-like network the gel was stained with

Congo Red. Congo Red it has largely been used as diagnostic tool for the detection of amyloid deposits and in general for detection of the presence of β-sheets[9, 10] as it has been shown to interleave between two strands of β-sheets in amyloid fibrils [11]. The stained sample, under cross-polarized filters, shows the apple-green color associated with the interleaving of the Congo Red to the β-sheets as well as birefringence. Thus the peptidic sequence contributes to the self-assembling proces by forming β-sheets (Figure 6).

Returning to the apparent dichotomy noted earlier, the DSC and XRD data suggest that the glass is amorphous while the microscopy and Congo Red dyeing data suggest the presence of ordered (liquid crystal and β-sheet) regions. Such a dichotomy has been noted in an earlier study of cyclosporine [7]. One explanation is that both DSC and XRD measure bulk properties (i.e. they have the resolution only to measure change on a relatively macroscopic scale) and hence neither is sensitive to order on a molecular scale. The microscopic and dyeing analyses, conversely, are sensitive to molecular structure and hence show evidence of the ordered phases. It is likely, therefore, that the glass formed in the DSC post melting is not truly amorphous but as liquid crystal and β-sheet regions.

3 CONCLUSIONS

Nanofibres peptide amphiphile gels are biocompatible materials with potential application in regenerative medicine and drug delivery. This report shows that electromagnetic radiation heating of peptide amphiphiles bearing acyl chains is a fast method of producing peptide nanofibres gels.

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