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Sol–gel transition of charged fibrils composed of a model amphiphilic peptide

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ABSTRACT

We characterized the sol–gel transition of positively charged fibrils composed of the model amphiphilic peptide RADARADARADARADA (RADA 16-I) using a combination of microscopy, light scattering, microrheology and rheology techniques, and we investigated the dependence of the hydrogel formation on fibril concentration and ionic strength. The peptide is initially present as a dispersion of short rigid fibrils with average length of about 100 nm. During incubation, the fibrils aggregate irreversibly into longer fibrils and fibrillar aggregates. At peptide concentrations in the range 3–6.5 g/L, the fibrillar aggregates form a weak gel network which can be destroyed upon dilution. Percolation occurs without the formation of a nematic phase at a critical peptide concentration which decreases with increasing ionic strength. The gel structure can be well described in the frame of the fractal gel theory considering the network as a collection of fibrillar aggregates characterized by self-similar structure with a fractal dimension of 1.34.

1. Introduction

The phase diagram of fibrils composed of peptides and proteins is involved in a large variety of problems in biology, biophysics and biochemistry: examples include the behavior of actin filaments, which are key components of the cytoskeleton [1–4], and of whey protein aggregates in the food industry [5–9]. Despite the phase behavior of rod-like colloids has been widely investigated in the literature [10–13], the study of the phase diagram of protein solutions is often complicated by the presence of irreversible aggregation and metastable phases [8]. As a consequence, a rich and dynamic phase behavior is often observed, with formation of liquid–crystalline phase, gels and phase separation [14–18].

One example of the importance of controlling the stability of protein aggregates is found in the biotechnology context, where protein-based drugs and functional materials are increasingly developed [19–22]. For instance, thanks to several advantages such as biocompatibility and degradability, hydrogels obtained from synthetic peptides have received large attention in fields ranging from 3D cell cultures [23] to drug release [24] and delivery [25,26], as well as tissue engineering and tissue repair [27]. During the industrial processing of the material, protein solutions are exposed to several stresses, such as shear forces and incubation at low pH, which can potentially induce the degradation of the product. Obviously, the control of the aggregation state of peptides and proteins during their manufacturing is a prerequisite for the successful application of the material [28,29].

The stability of protein fibril dispersions is involved also in biological systems, for instance in the aggregation of amyloidogenic peptides and proteins involved in several neurodegenerative disorders [30]. Amyloid fibrils associate in large bundles and plaques, which are eventually observed in the tissues of the patients. Lateral association of protofilaments in multistranded fibrils and laminate structures has also been observed with both globular proteins [31] and synthetic peptides [32]. However, the understanding of the interactions between fibrils and of the phase diagram of amyloid fibril dispersions is still limited.

In this work, we consider as a model system the stability behavior of positively charged fibrils composed of the RADARADARADARADA (RADA 16-I) peptide [33]. RADA 16-I is a well-known example of the family of self-complementary peptides which have been designed to self-assemble in a controlled way into fibrils and higher ordered structures depending on the pH value [34–36]. Ionic-complementary peptides are typically 8–16 amino acid long peptides whose structure displays hydrophobic residues on one side of the polypeptide chain and hydrophilic residues on the other side [20,37–39]. The self-assembly process is driven both by the hydrophobic double sheet formation inside the fibril and the

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electrostatic interactions between charged residues on the hydrophilic side [37]. The inter- and intramolecular forces determining the secondary structure and the aggregate state of the peptides can be finely tuned by a series of intrinsic properties (e.g. amino acid periodicity and charge distribution) and environmental conditions, such as pH, salt and ionic strength [20,40,41].

Here we apply a combination of microscopy, light scattering and rheology techniques to characterize the gelation process of RADA 16-I fibrils dispersions and the morphology of the resulting hydrogel at low pH. The fibril dispersions formed by RADA 16-I in water solutions at low peptide concentration under acidic conditions has been described in a previous work [42]. Here we show that the fibrils aggregate irreversibly into longer fibrils and fibrillar aggregates which at sufficiently large volume fractions form a reversible gel network which can be destroyed upon dilution. The phase transition occurs directly from the isotropic liquid to the gel phase without the formation of a nematic phase at a critical peptide concentration which decreases with increasing the salt concentration. We show how the dependence of the critical percolation concentration on the ionic strength can be well rationalized in the frame of the fractal gel theory.

2. Materials and methods

2.1. Material

The RADA 16-I (Ac-R-A-D-A-R-A-D-A-R-A-D-A-R-A-D-A-NHC-OCH3) peptide was provided by Lonza Ltd (Visp, Switzerland) as lyophilized powder in the form of trifluoroacetic or chloride salt. The material was used as received without further purification. Before each experiment the peptide was freshly dissolved in a suitable amount of solution. The concentration was checked by UV absorbance after mild stirring for 10 min for homogenization, and the pH value of the final solution was measured by a SevenEasy pH meter (Mettler Toledo). The results shown in this work refer to the peptide in the trifluoroacetic salt form. It is worth noting that very similar results have been obtained with peptides originating from three different production batches and with the peptide in the form of chloride salt [42].

2.2. Atomic force microscopy (AFM)

10 μL of RADA 16-I samples diluted 200-fold were deposited on a freshly cleaved mica surface for 30 s before washing with Milli-Q deionized water to remove unattached materials and gently drying under nitrogen flux. Samples were imaged at room temperature by an Asylum Cypher Scanning Probe Microscope (Asylum Research, Santa Barbara, CA, USA) operating in tapping mode. Scan rate of 1 Hz and antimony doped silicon cantilevers with resonance frequency in the range 300–360 kHz and tip radius of 8 nm (Veeco, Plainview, NY, USA) were used.

2.3. Dynamic light scattering (DLS)

Dynamic light scattering (DLS) measurements at a fixed angle ranging from 20° to 90° were performed *in situ* using a goniometer BI-200SM (Brookhaven Instruments, Holtsville, NY, USA) equipped with a solid-state laser, Ventus LP532 (Laser Quantum, Manchester, UK) with a wavelength $\lambda_0 = 532$ nm as the light source. The average hydrodynamic radius of the sample was calculated from the measured diffusion coefficient based on the Stokes–Einstein equation.

DLS was also measured at a fixed angle of $\theta = 173°$ using a Zetasizer Nano (Malvern, Worcestershire, UK) with laser beam of wavelength $\lambda_0 = 633$ nm.

2.4. Diffusing wave spectroscopy (DWS)

Diffusing Wave Spectroscopy measurements were done using a LS Instrument (Fribourg, Switzerland). Standard reference polystyrene particles with nominal hydrodynamic radius of 190 nm were mixed with the peptide solution at particles concentration of 2 g/L. Glass cuvettes with 0.2 cm light path were used (Hellma, Müllheim, Germany).

2.5. Rheology

Rheology experiments were performed using an ARES Rheometer (Rheometric Scientific, Piscataway, NY, USA) working under oscillatory, strain-controlled mode equipped with 25 mm cone-plane geometry. The gel was gently introduced between the cone and the plate and 10 min of re-equilibration were allowed before starting the measurements. Before measuring the frequency-dependent loss and storage modulus, the strain-dependent moduli were measured in a strain amplitude range from 0.1% to 200% at constant frequency of 200 rad/s in order to determine the linear viscoelastic region. Frequency-dependent measurements were performed at constant strain amplitude values ranging from 30% to 40% and within a frequency range of 0.05–500 rad/s. All measurements were performed at 25 °C at five different RADA 16-I concentrations (4.5, 5, 7, 9 and 10 g/L) dissolved in 10 mM HCl with 50 mM NaCl at pH 2.0.

2.6. Phase diagram

The phase diagram of RADA 16-I fibril dispersions at 25 °C was investigated as a function of the fibril concentration in the range 0.4–6.5 g/L and as a function of NaCl concentration in the range 0–200 mM. The possible presence of nematic phase was checked by observation under cross-polarized light using an optical microscope Zeiss Axioskop 2 (Carl Zeiss Jena GmbH, Jena, Germany) as described in Jung et al. [43]. The concentration of the RADA 16-I fibrils in the dispersion has been evaluated by subtracting from the total weighted peptide concentration the soluble monomer fraction, which has been quantified by Size Exclusion Chromatography as described in our previous work [42].

3. Results and discussion

3.1. Kinetics and mechanism of gel formation

We start our analysis by investigating the aggregate state of the peptide at concentration of 10 g/L in 10 mM HCl solutions at pH 2.0. After re-dispersion of the peptide powder, a transparent solution is observed. Atomic force microscopy pictures of samples taken few minutes after re-dispersion show the presence of a rather homogeneous population of fibrils with an average length of about 100 nm (Fig. 1a). In a recent work we have shown by FTIR and CD assays that these fibrils contain β-sheet structures [42], and that AFM images are consistent with TEM pictures [42].

The kinetic stability of the fibril dispersion was followed *in situ* by Dynamic Light Scattering (DLS) with both the Malvern Zetasizer Nano and the Brookhaven instrument by monitoring the relative changes in the average size with respect to the initial condition. As it can be seen in Fig. 1b the results obtained with the two instruments are very similar. In addition, we verified that the time evolution of the average $< R_h >$ relative to the initial value is independent of the scattering angle (see Supplementary Material Fig. S1).

As shown in Fig. 1b, the fibril dispersion is not stable during time. The time evolution of the average hydrodynamic radius