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Influence of the chirality of short peptide supramolecular hydrogels in protein crystallogenesis†

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For the first time the influence of the chirality of the gel fibers in protein crystallogenesis has been studied. Enantiomeric hydrogels 1 and 2 were tested with model proteins lysozyme and glucose isomerase and a formamidase extracted from B. cereus. Crystallization behaviour and crystal quality of these proteins in both hydrogels are presented and compared.

Chirality is ubiquitous in nature and has major implications in cell interactions and biological processes. Within this topic, chiral discrimination on chiral surfaces has significant effects in protein adsorption and cellular adhesion and proliferation.1 It would be expected that similar chiral discrimination was feasible with other common macromolecular hydrogels, which either have no stereocenters (polyacrylamide) or are constituted diastereoisomERICALLY with proteins, thus expecting different behaviors. It is worth noting that the structural simplicity of short peptides is essential for this purpose, allowing an easy access to their corresponding enantiomers. This property is not feasible with other common macromolecular hydrogels, which either have no stereocenters (polyacrylamide) or are constituted by the natural chiral product (agarose).

To test this hypothesis we selected two cysteine-based peptides, compound 1 (N,N'-di(benzoyl)-L-cysteine diamide) and its corresponding D enantiomer 2 (N,N'-di(benzoyl)-D-cysteine diamide) (Fig. 1) due to their capacity to self-assemble in neat water to give the corresponding hydrogels.⁷ They were tested with two model proteins, chicken HEWL lysozyme and glucose isomerase, and one target protein, a formamidase extracted from B. cereus being the first time that supramolecular peptide based hydrogels are used for this purpose. Additionally, the results were compared with crystal

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† Electronic supplementary information (ESI) available: General experimental details, 1H NMR and 13C NMR of hydrogels 1 and 2, VT-NMR and DSC spectra of hydrogels 1 and 2 and crystallization and crystallography data. See DOI: 10.1039/c4cc09024a

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Fig. 1 Characterization of hydrogels 1 and 2 by TEM microscopy, circular dichroism and rheology. TEM images of negatively stained dried hydrogels at 3 mM: (A) 1, (B) 2. Scale bar at 0.2 μm. (C) CD spectra and (D) rheology of hydrogels 1 and 2.
growth in agarose gels to investigate their potential in protein crystallization.

Compounds 1 and 2 were selected from a family of 22 cysteine derivatives based on its capacity to form a stable and completely transparent hydrogel. TEM images of hydrogel 1 showed self-assembled nanofibers of diameters ranging from 10 to 20 nm and lengths that go up to μm in size (Fig. 1). Fibrous aggregates co-exist with regions where fibers are more dispersed. Hydrogel 2 presented fibers of the same aspect. To gain insight into the molecular arrangement of these hydrogels circular dichroism (CD) spectra were recorded (Fig. 1). Hydrogel 1 exhibited a negative band near 218 nm (π* transition), a positive band near 250 nm (π* disulfide band and/or π* of the aryl groups) and a negative band near 280 nm (π* of the aryl groups) in good agreement with the CD spectrum from other oligopeptide hydrogels identified as the β-sheet conformations. The CD spectrum of hydrogel 2 is the specular image of hydrogel 1 showing that the chirality of the peptides is conserved and transferred supramolecularly to the fibers. Hydrogels 1 and 2 were also analyzed using a regime of simple oscillatory shear stress through frequency (see ESI†) and amplitude sweep tests. Both hydrogels showed G′ values (elastic modulus) higher than G″ values (viscous modulus) indicating the predominant elastic behavior of the gels (Fig. 1). Based on the magnitude of the values of G′, G″ and σc (G′ ≈ 30 Pa; G″ ≈ 4 Pa; σc < 1 Pa) we can conclude that these hydrogels are extremely weak. The similar values of G′ and G″ for hydrogels 1 and 2 confirm that both enantiomeric hydrogels have the same rheological properties. VT-NMR studies on both hydrogels showed that the signals of the aromatic region were shifted 0.5 ppm downfield as the temperature increased from 25 °C to 70 °C. The small values of T2 (1.84 s for the 7.74 ppm signal) and the negative values of noe indicated that the 1H-NMR signals corresponded to aggregates of the di-peptide which are not higher enough to be NMR-silent. The stability of these aggregates indicated that Tg should be higher than 70 °C. In fact in the 75/25 H2O/DMSO mixture, the reported value of Tg for 1 was above 90 °C. DSC scans in both directions of hydrogels and 2 did not show any endothermic or exothermic peaks. These results indicated continuous gel to sol and sol to gel transitions (see ESI†).

The two enantiomeric hydrogels 1 and 2 were then tested as the supramolecular media for protein crystallization. Following the procedure described in ESI† protein was allowed to diffuse in the gel prior to the addition of the precipitant. Fig. 2A shows a typical counter-diffusion pattern of lysozyme crystals grown in hydrogel 1. The effect of hydrogels 1 and 2 on the quality of lysozyme crystals was evaluated from a set of lysozyme crystals prepared at beam-lines Xaloc (ALBA) following the standard quality criterion.11

Lysozyme crystals grown in hydrogel 2 were better than those obtained in hydrogel 1, reaching the resolution limit of the detector (0.95 Å) and having the best quality indicators in terms of I/σ(I), Rmerge and mosaicity (Fig. 2B). The excellent reproducibility of crystals grown in hydrogel 2 (Fig. S1 and Table S2, ESI†) is also worth mentioning. Moreover crystal quality indicators of crystals grown in agarose (able to produce protein crystals of high quality) were similar to those obtained in hydrogel 1 but still inferior to those in hydrogel 2. The agarose gel has been proven to facilitate the soaking of different compounds including cryoprotectants. In order to evaluate the possible cryo-protection capacity of these new hydrogels we compared the crystal quality of cryo-protected and “naked” (flash frozen without cryoprotectants) lysozyme crystals grown in hydrogel 1 and agarose from two data sets collected at beam-lines ID23 and BM30 (ESRF). It is important to notice that the polymeric nature of both agarose and hydrogel 1 allowed us to collect full data sets without the need to use any additive (Fig. S2, S3 and Table S3, ESI†). A similar analysis was performed with glucose isomerase crystals grown in hydrogels 1, 2 and agarose. In this case crystals obtained in hydrogel 1 and agarose were more homogeneous and of better quality than those grown in hydrogel 2 (Fig. 2C, Table S4 and Fig. S4, ESI†). However, a second crystal form (P212121) was identified only in the experiments run with hydrogel 2 that co-exist with the other polymorph (P2221), the form normally found under similar crystallization conditions. Dauter and co-workers have described a new crystal form (P212121) of glucose isomerase and have solved its structure (PDB ID 1OAD).11,12 Remarkably, the polymorph found in hydrogel 2 presents different unit cell parameters (Å): 86.00, 93.68, 99.22 versus 98.45, 129.59, 78.33 for a, b and c, respectively (Fig. S4, ESI†). To date there has been only one PDB entry (PDB ID 1O1H) with a similar unit cell and space group but obtained under different crystallization conditions.

The quality of this primitive orthorhombic crystal was the highest of all glucose isomerase crystals tested, with the structure determined at 1.20 Å and refined to R/Rfree values of 10.73/13.22 (%) (Table S5, ESI†). When compared with the model used for molecular replacement (PDB. ID 1OAD) the deviation of the main chain
is minimal (rmsd of 0.3). It is worth mentioning that this new polymorph has only been obtained in free solution using other precipitants, as in the case of PDB ID. 101H that crystallized using MPD, or in the presence of high agarose concentration. In our case, under identical crystallization conditions, the inhibition of nucleation while crystals grown in agarose were of low quality proving the different behavior of these hydrogels.

To sum up, we present the novel use of supramolecular hydrogels based on short-peptides in neat water for protein crystallization. These hydrogels, made of symmetrical L and D di-cysteine derivatives, were used to grow protein crystals of high quality. Differences in crystal quality and packing for the three proteins have been observed using enantiomeric hydrogels 1 and 2, suggesting a significant influence of the fibers’ chirality in the crystallization process. The formation of the orthorhombic polymorph of glucose isomerase and the growth of the highest quality crystals of formamidase exclusively in hydrogel 2 support this hypothesis. This fact highlights the relevance that chirality may have in protein crystallogenesis for X-ray structural determination. Moreover these supramolecular peptide based hydrogels are excellent mediators for protein crystallization producing lysozyme and glucose isomerase and formamidase crystals with excellent quality indicators. Peptide based hydrogels also acted as cryo-protectants avoiding the use of additives. Hence, these novel chiral gels would expand the field of protein crystallogenesis.

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Notes and references


