Oligo-Urea with No Alkylene Unit Self-Assembles into Rod-Like Objects in Water

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ABSTRACT: Long and rigid objects formed by self-assembly in water are useful as templates or for their rheological or biological properties. They are usually obtained by combining hydrogen bonding and strong hydrophobic interactions brought by an alkyl or alkylene chain. We report a simple access to well-defined rod-like assemblies in water based on a penta-urea sticker directly connected to PEO side chains. These assemblies are characterized by an average length of several hundreds of nanometers and a monodisperse radius (4.5nm) resulting from a reduced lateral aggregation of the stickers.

1. Introduction

One-dimensional assemblies (e.g. actin, microtubule, collagen) are ubiquitous in Nature and show a rich variety of functions. Therefore, the preparation of rod-like objects by self-assembly of synthetic building blocks in aqueous medium has been a major objective in the past decade.[1] These anisotropic systems offer a huge potential for alignment or percolation, and have been used to form hydrogels,[2] or as templates for various materials,[3] as well as for their biological properties.[4]

A stable anisotropic structure can only form if it has a thermodynamic advantage versus competing assemblies, and several concepts have been demonstrated to allow significant selectivity. In the case of block-copolymers[5] or amphiphiles that form worm-like micelles,[6] the anisotropy simply results from the favorable volume fraction of the hydrophobic part. It is however possible to go beyond this geometrical effect by introducing anisotropic interactions such as in crystallization-driven self-assembly of amphiphilic block copolymers where the assembly is guided by the crystallization of the hydrophobic blocks[7] or in rod-coil molecules[8] where the rigid hydrophobic cores tend to pack in a parallel fashion to maximize van der Waals and pi-stacking interactions. In this respect, the directionality of hydrogen bonds (Hbonds) is particularly useful, as demonstrated in the case of beta-sheet forming peptides[9] and nanotube forming cyclic peptides.[10] In another approach, Hbonds and hydrophobic interactions have been combined in peptide amphiphiles:[11] a hydrophobic alkyl chain provides a strong driving force for assembly, but the anisotropy is controlled by the Hbonds between peptides. This synergy between hydrophobic interactions and Hbonding has also been successfully applied to many non-peptidic bolaamphiphiles with benzene tricarboxamide,[12] bis-urea[13] or bis-squaramide[14] stickers associated to alkylene units. In fact, in these systems, the absence of the alkylene part has been shown to result in no assembly, because of the strong competition of water molecules for the Hbond stickers.[13a] As a result, apart from peptides, all these anisotropic systems that form

in water by Hbonding also contain a hydrophobic alkyl or alkylene part. Intrigued by the counterexample provided by beta-sheet forming peptides, we wondered whether the loss of the alkylene part could be compensated by increasing the number of Hbonding units and whether this simplified design would bring some particular advantage.

2. Results and discussion

2.1. Synthesis

In order to test the influence of the number of Hbonds, PEO3U, PEO4U and PEO5U were synthesized with poly(ethylene oxide) chains directly connected to a sticker composed of 3, 4 or 5 urea groups, respectively (Scheme 1, Table 1 and Supporting Information (SI) §1.2).
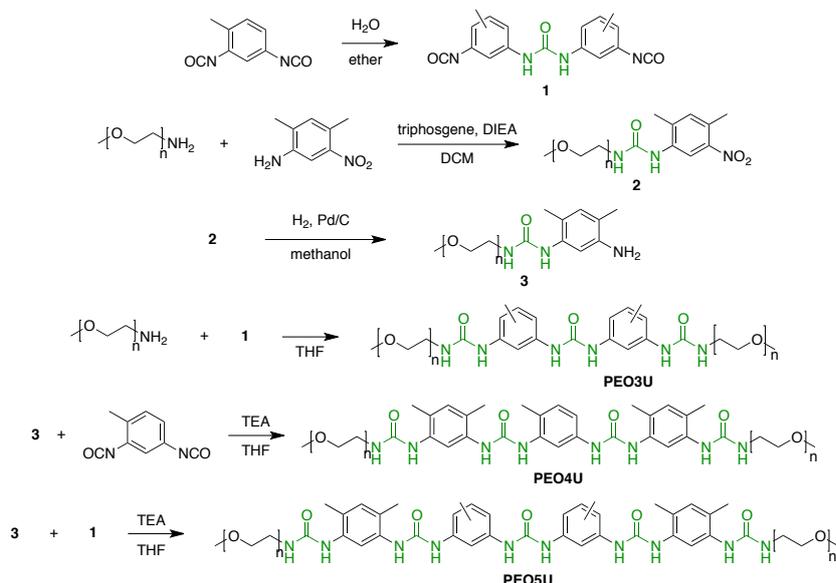
Scheme 1. Synthesis of PEO3U, PEO4U and PEO5U (n ~ 50)

Table 1. Characteristics of unimers[a,b] and particles[c] obtained by direct dissolution in water (see SI).

polymer	M_n (NMR) (kg/mol)[a]	M_n (SEC) (kg/mol)[b]	Đ SEC[b]	M_w (kg/mol)[c]	N_{agg}[c,d]	R_g (nm)[c]	R_h (nm)[c]
PEO3U	5.1	6.1	1.2	4.7 +/- 0.4	~ 1	< 20	2.0 +/- 0.5
PEO4U	5.1	6.8	1.1	85 +/- 10	16 +/- 2	< 20	10 +/- 1
PEO5U	5.0	7.0	1.1	2600 +/- 200	450 +/- 30	28 +/- 4	20 +/- 1

[a] ^{1}H NMR in DMSO-d_6. [b] SEC in DMF (PMMA standards). [c] PEO3U, PEO4U and PEO5U were dispersed directly in water respectively at 5, 20 and 0.2g/L. The values of M_w, N_{agg}, R_g and R_h are true values (i.e. concentration independent). [d] Aggregation number: $N_{agg} = M_w/M_{w,unimer} = M_w/(M_n(NMR) \times Đ)$.

2.2. Influence of the preparation pathway

The three polymers differing solely by the number of urea units per molecule, were initially dispersed in pure water. PEO3U and PEO4U afforded transparent solutions upon direct dispersion up to at least 10g/L, whereas PEO5U solutions were only transparent up to 1g/L. Static and dynamic light scattering (Table 1 and SI §3) revealed that PEO3U did not self-assemble in water but remained as unimers. PEO4U solutions hardly aggregated, and PEO5U self-assembled into very large particles.

The direct dispersion method was then compared to other preparation pathways in order to determine whether the particles formed by PEO4U and PEO5U were under thermodynamic equilibrium. For PEO5U, the extent of aggregation depended on the preparation pathway, revealing that this polymer self-assembles in water as out-of-equilibrium structures. The extent of aggregation dramatically increased for PEO5U when it was first dissolved in DMSO followed by slow addition of water down to a DMSO/water content of 1/99 (vol/vol) (Figure 1a). Considering that PEO5U was dissociated in DMSO (the scattered intensity was extremely low in this solvent), this result reveals that self-assembly of PEO5U was promoted by the addition of water, but in a kinetically controlled way leading to a better organization using the "DMSO-route" than by direct dispersion in water (see §3.6. of the SI for more details). This result infers that although no alkyl chain is present in PEO5U, hydrophobic interactions probably still play a significant role in its self-assembly.

For PEO4U, the light scattering data could not be interpreted assuming only one monodisperse population of scatterers because their dimensions would be extremely large compared to their extent of aggregation. Based on the rather low value of M_w, it was concluded that PEO4U only weakly aggregated following the DMSO-route, leading to a solution containing rather small particles (possibly unimers) mixed with a few larger particles responsible to the rather large R_g value (see SI §3.7).

To summarize, upon dispersion in water, PEO3U formed unimers, PEO4U aggregated weakly, and PEO5U self-assembled strongly. Moreover, the extent of self-assembly was promoted by the DMSO-route. The rest of the paper therefore focuses on the large supramolecular assemblies formed by PEO5U in DMSO/water 1/99 v/v.
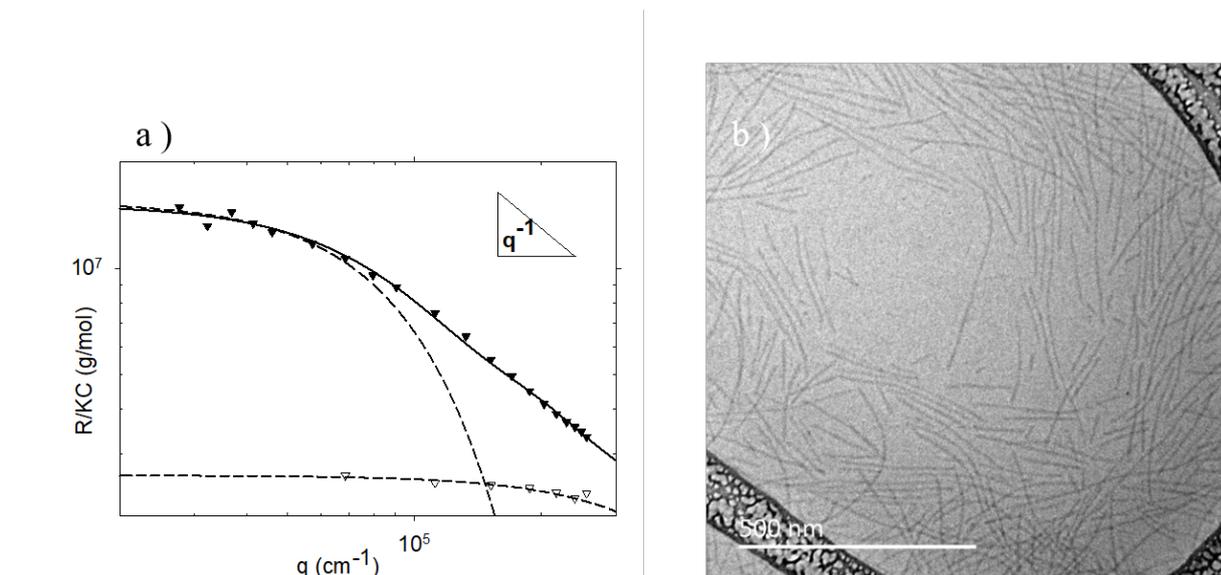
Figure 1. a) Normalized scattered light intensity versus wavevector for PEO5U prepared by direct dissolution in water (0.5g/L) ($\triangledown$) or following the DMSO-route (1g/L in DMSO/water 1/99) ($\blacktriangledown$) (SI §2). Continuous line: fit with a model for cylinders (molecular weight M_w = 1.5x10^{7}g/mol, length L = 530nm, radius r = 4.5nm) (SI §3.4). Dashed lines: Guinier analysis (only relevant when q.R_g < 1). For the direct dispersion in water, M_w = 2.6x10^{6}g/mol and R_g = 28nm, see Table 1. For the DMSO-route, M_w = 1.5x10^{7}g/mol and R_g = 150nm. b) CryoTEM image of PEO5U prepared following the DMSO-route (DMSO/water = 1/99, SI §2.2).

2.3. Quantitative characterization of PEO5U in DMSO/water 1/99 v/v

CryoTEM (Figure 1b) unambiguously revealed that the DMSO-route led to the formation of long (hundreds of nanometers) and rigid rods, polydisperse in length but quite monodisperse in radius. The average radius of the cylinders was estimated to be r = 4.5nm. This value lies between the R_h of PEO3U unimers in water (R_h ~ 2nm) and a fully extended PEO chain (~DP$_n$

x 0.28nm = 14nm)[15] and is therefore compatible with the size of the PEO5U elementary brick. It is also in perfect agreement with the radius estimated from Small Angle Neutron Scattering experiments (See SI §4).

Treatment of the low-q data of the static light scattering (SLS) experiments with a Guinier model indicated that the particles formed by PEO5U following the DMSO-route were strongly aggregated: R_g = 150nm and M_w = 1.5x10^{7}g/mol (see Figure 1 and SI §3.4). The high-q data (q > 8x10^{4}cm^{-1}) revealed a q^{-1}-dependency of the scattered light intensity, in agreement with the observation of long and rigid rods in cryoTEM (Figure 1b).[16] The SLS data were thus more quantitatively analyzed using a model of monodisperse cylinders[17] (see SI §3.4). Since the q-range accessible with light scattering did not allow the observation of the region sensitive to the radius r of the cylinders (< 10 nm), the data were treated by fixing r = 4.5nm, according to the cryoTEM and SANS results (see SI §4). The best fit yielded a molecular weight M_w = 1.5x10^{7}g/mol and a length L = 530nm, the latter value being in agreement with the cryoTEM micrographs and the value of R_g (R_g = L/√12 = 150nm for infinitely thin cylinders with r << L).[18] From the q^{-1} dependency of R/KC at the highest q values accessible by SLS, the mass per unit length M_L of the cylinders could be deduced from (R/KC).q = M_L.π (Figure S11).[19] M_L = 2.6x10^{4}g.mol^{-1}.nm^{-1} was found which is in excellent agreement with M_w/L = 2.8x10^{4}g.mol^{-1}.nm^{-1}, confirming the consistency of the model used. It was previously determined that the distance between two Hbonded bis-ureas is 0.46nm.[20] Assuming that the distance between two H-bonded PEO5U units is the same, M_L should be equal to M_L(stack) = $M_{w,unimer}$/0.46 = 1.2x10^{4}g.mol^{-1}.nm^{-1} if the PEO5U units are simply stacked one on top of the other with Hbonds forming between them. Considering that M_L(stack) is about half of the experimental M_L found, we conclude that about two PEO5U molecules can be found in the cross-section of the cylinders.

The normalized autocorrelation functions obtained from dynamic light scattering (DLS) measurements were analyzed in terms of a relaxation time (τ) distribution. A single, slightly polydisperse, relaxation mode was observed for all the scattering wavevectors (Figure S8b).

However, $\Gamma = <1/\tau>$, the intensity-averaged relaxation frequency of the auto-correlation function was shown not to follow a q^2-dependence along the whole q-range indicating a complex diffusion process (Figure S9). The diffusion coefficient determined by DLS, D_c, showed a q^2-independence at low wavevector where the particles are probed at large observation scale. The apparent hydrodynamic radius (R_{ha}) was thus estimated from the mean value of D_c at low wavevector according to the Stokes-Einstein equation, giving R_{ha} = 80nm. The hydrodynamic radius of rigid rods is related to their length L and their radius r according to equation (1)[21].

$$R_{ha} = \frac{L}{2\sigma - 0.19 - \frac{8.24}{\sigma} + 12/\sigma^2} \qquad (1)$$

where L is the length of the cylinder and $\sigma = \ln(L/r)$, with r the radius of the cylinder.

Using equation (1) and r = 4.5nm estimated from cryoTEM and SANS, L = 700nm was found for PEO5U. Thus, the DLS results confirm the SLS and cryoTEM data.

Hydrophobic interactions

Hydrogen bonding + hydrophobic interactions along the axis of the cylinder
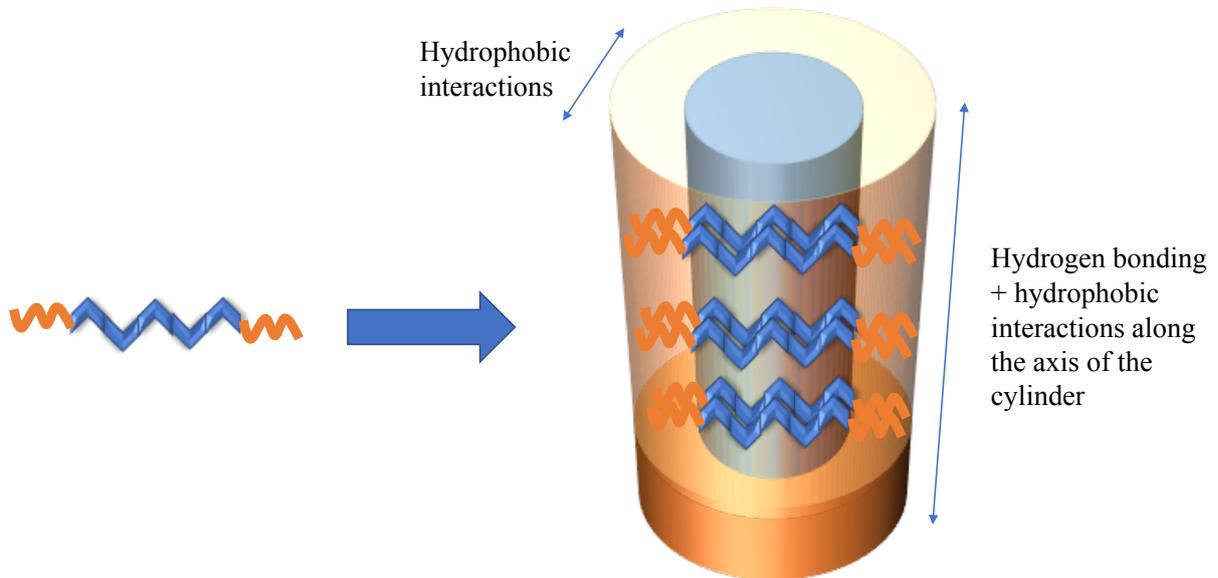
Figure 2. Tentative model for PEO5U self-assembly.

With the exception of peptides, molecules that self-assemble into 1D structures in water through Hbonds rely on alkyl or alkylene parts to protect the Hbonds from water through strong hydrophobic interactions.[9-14] Usually, the Hbonds have been assumed to form along the rod

axis, while hydrophobic interactions caused lateral aggregation of 5[13a] or 10[14,22] molecules within the cross-section. In spite of the absence of alkyl/alkylene spacers, the self-assembled cylinders formed by PEO5U are very long and their characteristics are also consistent with a model where the Hbonds form along the rod-axis. Lateral aggregation is however strongly reduced (2 molecules in the cross-section) compared to previously reported systems (Figure 2). This reduced aggregation may be due to limited hydrophobic interactions or to the particular topology of the stickers. It is also of interest to compare the structure of the objects formed by PEO5U to a recently reported cyclic peptide-poly(ethylene glycol) conjugate.[23] Both systems form rod-like objects in water and are composed of a central Hbonded sticker (8 Hbonds versus 10 in the case of PEO5U) decorated by two PEG chains of the same size (2000g/mol). However, the aggregation number of the cyclic octapeptide is much smaller than in the case of the penta-urea (22 versus 2000).

3. Conclusions

We report a simple access to rod-like assemblies in water based on a penta-urea sticker decorated with PEO side chains. With the exception of peptides, this example is the first where the assembly is promoted by Hbonding aromatic stickers directly in water in the absence of alkyl chains or alkylene spacers to protect the Hbonds from the competitive water molecules. Our cryoTEM, SLS, DLS and SANS results indicate that the formation of 1D structures is possible in water in spite of the competitive nature of the solvent and of the PEO arms. PEO3U and PEO4U hardly self-assemble in water even though they could form respectively 6 or 8 Hbonds per molecule, but PEO5U forms well-defined rods, hundreds of nanometer long and with a monodisperse radius. These pathway-dependent, out-of-equilibrium rods can conveniently be prepared using DMSO as an initial disruptive solvent. Finally, a weak lateral aggregation was noticed, with only two molecules in the cross-section compared to 5 or 10 for other systems.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author

Acknowledgements

Keywords

self-assembly, hydrogen bond, rod-like nanoparticle, light scattering

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