Programmed assembly of expanded rigid-rod β-barrels by supramolecular preorganization

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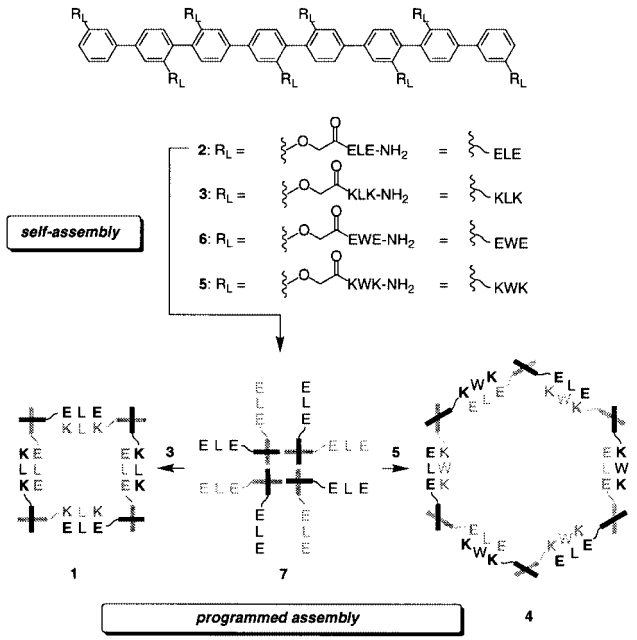
A simple strategy for (a) expansion and (b) variation of the internal chemical nature of rigid-rod β-barrels is reported, and the importance of supramolecular preorganization for their programmed assembly is discussed.

In nature, barrel-like folds[1] serve to recognize antigens[2] and secondary metabolites,[3,4] to mediate transport across biomembranes,[5] to catalyze enolate chemistry,[2,6] and to let jellyfish fluoresce.[7] In view of this functional diversity in nature, it is surprising to note that the design of artificial barrels has attracted little scientific attention. According to Fujita and coworkers, the assembly of nanotubes[8–11] with precisely defined length but flexible diameter in isotropic media, *i.e.* 'nanobarrels', was achieved last year for the first time.[12,13]† More recently, we have used the unique properties of rigid-rod molecules to create tetrameric barrel **1** in detergent-free water by programmed assembly of the complementary tripeptide-rods **2** and **3** (Scheme 1).[14] Rigid-rod β-barrel **1** is characterized by a length of 3.4 nm and an internal diameter of *ca.* 0.8 nm.[14] Constructive electrostatic interactions between lysine and glutamate residues account for the hydrophilicity of the barrel surface, while leucine residues produce a hydrophobic interior that can accommodate planarized β-carotene.[14] Several design strategies for further expansion of rigid-rod β-barrels are conceivable.[15] Here we report programmed assembly of the hexameric rigid-rod β-barrel **4** in detergent-free water. In this case, barrel expansion was accomplished by partial replacement of the internal leucine residues in tetramer **1** by the more bulky tryptophan residues in hexamer **4** (Fig. 1). We further provide experimental support that supramolecular preorganization may play an important general role in the programmed assembly of rigid-rod β-barrels in water (Scheme 1).



Scheme 1 Programmed assembly of rigid-rod β-barrels **1** and **4** by supramolecular preorganization with pinwheel **7** in water, pH 6.4. One-letter abbreviations for amino acids are used (E: glutamate, K: lysine, W: tryptophan, L: leucine). All supramolecules are displayed schematically in axial view with the benzene rings as bold lines. The first 'layer' of arene-tripeptides is in black, the second in grey, and the following six not shown for clarity. Amino acids with residues located at the outer barrel surface are in bold, those at the inner surface not.

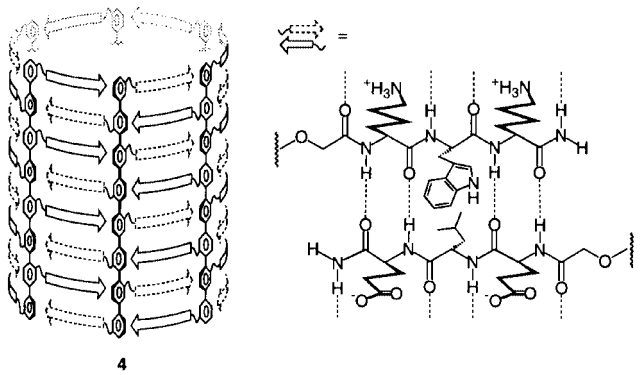


Fig. 1 Structure of hexameric rigid-rod β-barrel **4**. Amino acid residues located at the outer barrel surface are in bold, those at the inner surface not.

For programmed assembly of expanded rigid-rod β-barrels, we synthesized and characterized the complementary KWK-rod **5** and EWE-rod **6** following the experimental procedure for ELE-rod **2** and KLK-rod **3** that has been published in detail.[14] Size exclusion chromatogram (SEC) and circular dichroism (CD) spectrum of KWK-rod **5** in detergent-free water, pH 6.4, supported the previous observation with KLK-rod **3**[14] that cationic tripeptide-rods are monomeric under these conditions (Fig. 2, dashed line). Increasing amounts of ELE rod **2** converted the CD spectrum of KWK-rod **5** into that typical for rigid-rod β-barrels,[13–15] *i.e.* **4** (Fig. 2, continuous line). Namely, low energy negative (341 nm, $\Delta\varepsilon$ −5.8) and positive (294 nm, $\Delta\varepsilon$ +2.0) CD Cotton effects (CEs) centered around the octa(*p*-phenylene) ^{1}L transitions (319 nm, ε 28.6 mM^{-1} cm^{-1}) are followed by weaker negative and 'positive' CD CEs (253 nm, $\Delta\varepsilon$ −4.0; 242 nm, $\Delta\varepsilon$ −0.5) and two strong high energy CD CEs (232 nm, $\Delta\varepsilon$ −12.5; 216 nm, $\Delta\varepsilon$ +8.8; not shown). The circular dichroic absorption at 341 nm increased with decreasing mole fraction x = [**5**]/([**5**] + [**2**]) until a maximal $\Delta\varepsilon$ of −5.8 was reached at x = 0.5 [Fig. 2, inset (a)]. This corroborated the expected 1 : 1 stoichiometry of rigid-rod β-barrel **4**. An identical CD spectrum at x = 0.5 was obtained by addition of KWK-rod **5** to ELE-rod **2** [Fig. 2, inset (b)]. The nonlinear mixing curve suggested that β-barrel formation is inhibited at $x < 0.33$ [Fig. 2, inset (b)]. Addition of ELE-rods **2** to preformed barrel **4** (x = 0.5) did not, however, cause barrel deconstruction at $x < 0.33$ [Fig. 2, inset (c)].

In water, ELE-rods **2** self-assemble into the tetrameric pinwheel **7** (Scheme 1).[16] These supramolecules are composed

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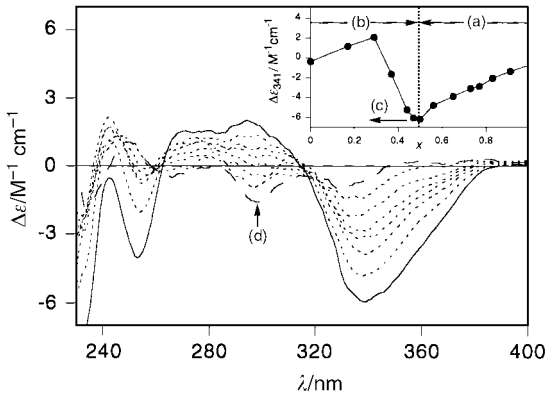

Fig. 2 Circular dichroism spectra of KWK-rod **5** (----) in the presence of increasing (····) and equimolar amounts of ELE-rod **2** (—). Inset: mixing curve for β-barrel **4**; x = [**5**]/([**5**] + [**2**]). (a) Addition of ELE-rod **2** (= pinwheel **7**) to KWK-rod **5** (identical with the displayed curves). (b) Addition of KWK-rod **5** to ELE-rod **2** (= pinwheel **7**). (c) Addition of ELE-rod **2** (= pinwheel **7**) to KWK-rod **5** (= barrel **4**) did not significantly change the initial CD spectrum (—). (d) The Cotton effect at 298 nm ($\Delta\varepsilon$ −1.1) was not observed in other peptide-rods[13–15] and is expected to originate from the L_a transition of the indoles in KWK-rod **5**. All experiments were performed in phosphate buffer (10 mM) at pH 6.4 with oligo(*p*-phenylene) concentrations around 10 μM, using a JASCO-710 spectropolarimeter. $\Delta\varepsilon$ values refer to the respective total octa(*p*-phenylene) concentration.

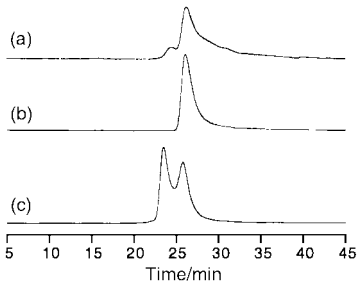

Fig. 3 Size exclusion chromatograms of (a) EWE-rod **6**, (b) ELE-rod **2** (= pinwheel **7**) and (c) β-barrel **4** (x = 1.0) with subsequently added ELE-rod **2** (= pinwheel **7**, final concentrations: [**2**]/[**5**] = 1.5/1.0). Chromatogram (b) displays new experiments that qualitatively reproduce previously reported data.[16] All experiments were performed in phosphate buffer (10 mM) at pH 6.4 with oligo(*p*-phenylene) concentrations around 10 μM, using a Superdex® 75 HR 10/30 prepacked column from Pharmacia Biotech (MW 70 000–3000, 1 mL buffer min^{-1}) coupled with a Jasco PU-980 pump and a Jasco UV-970 UV–VIS detector.

of a core of (presumably) edge-to-face π,π-stacked oligo(*p*-phenylene)s that is surrounded by anionic peptide strands in random-coil conformation.[16] The sharp peak of pinwheel **7** at R_t = 25.8 min [Fig. 3(b)] was used as a reliable internal standard for the determination of the molecular weight of barrels **4** by SEC [Fig. 3(c)]. Comparison of oligo(*p*-phenylene) and tryptophan absorption maxima at 319 nm and 282 nm,[17] respectively, confirmed that only the peak at R_t = 23.5 min [Fig. 3(c)] contained KWK-rod **5**. Rigid-rod β-barrel **4** is thus a hexamer under these conditions.

Programmed assembly of rigid-rod β-barrels using EWE-rod **6** instead of ELE-rod **2** was unsuccessful. Mixing of EWE-rod **6** with KWK-rod **5** resulted in immediate precipitation, and mixing with KLK-rod **3** did not result in clearly detectable supramolecular organization. The latter finding was of interest, because this non-forming barrel (composed of EWE-rods **6** and KLK-rods **3**) is the structural isomer of hexamer **4** (composed of ELE-rods **2** and KWK-rods **5**). This counter-intuitive result thus suggested that the (presumably) perpendicularly zigzagged preorientation of the lateral tripeptide strands of **2** in pinwheel **7** may significantly support the programmed assembly of rigid-rod β-barrels in water (Scheme 1). We further noted that the apparently reduced supramolecular preorganization in self-assembled EWE-rods **6** [Fig. 3(a) *cf.* ELE-rods **2** in Fig. 3(b)] and the capacity of pinwheel **7** to bind two KWK-rods **5** without significant suprastructural transformation [Fig. 2, inset (b), x = 0.33] are consistent with this view.

In summary, we have described the design and synthesis of expanded rigid-rod β-barrel **4** and obtained experimental support for the importance of supramolecular preorganization for programmed barrel assembly in detergent-free water. Compared to tetramer **1**,[14] hexamer **4** is of particular interest with regard to its enlarged hydrophobic interior (d > 2.0 nm in molecular models) and tryptophan residues at the inner barrel surface. We are currently interested in the influence of these 24 intratoroidal indoles in hexamer **4** on encapsulated chromophores of biological relevance.[3,4,7,18]

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Notes and references

† Interestingly, the design of artificial barrels in liquid crystalline media is much more developed because of their possible function as ion channels. See, *e.g.* S. Otto, M. Osifchin and S. L. Regen, *J. Am. Chem. Soc.*, 1999, **121**, 7276; 10 440.

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