Artificial Protein Block Copolymers Blocks Comprising Two Distinct Self-Assembling Domains

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Synthetic block copolymers comprising two or more compositionally distinct chains have attracted significant attention due to their ability to self-assemble into ordered microstructures.[1] Although tremendous progress has been made in the chemical synthesis of polymers, the unsurpassed degree of control and diversity of monomers[2] combined with advances in recombinant DNA technology permits the synthesis of unique artificial protein-derived block polymers. These include silk–elastin,[3] elastin–elastin hybrids of varying elastin blocks,[4] and helix–random coil–helix triblock combinations.[5] These polymers consist of nearly similar self-assembling chains, as in the case of silk–elastin and elastin–elastin hybrids, or one self-assembling motif fused to a disordered random motif. Herein, we describe three block copolymers comprising two distinct self-assembling chains—elastin (E) and cartilage oligomeric matrix protein coiled-coil (COMPcc; C)—fused in two orientations (EC and CE) and a final construct in which an additional E block is appended (ECE; Figure 1 A–C). Remarkably, the polymer structures as well as temperature and small-molecule-dependent assembly rely on the block orientation and the number of blocks.

Elastins consist of pentapeptide $(VPGXG)_n$ repeating units, where X is an interchangeable amino acid, that self-assemble into helical β-spirals.[6,7] Elastins exhibit a lower critical solution temperature (T_t) behaviour that can be tuned by varying the identity of X and the number of repeats (n).[8] By contrast, COMPcc self-assembles into a homopentamer of parallel α-helical coiled-coils to produce a hydrophobic pore that is 7.3 nm long with a diameter of 0.2–0.6 nm.[9] Individually, the E and C domains exhibit unique modes of self-assembly and conforma-
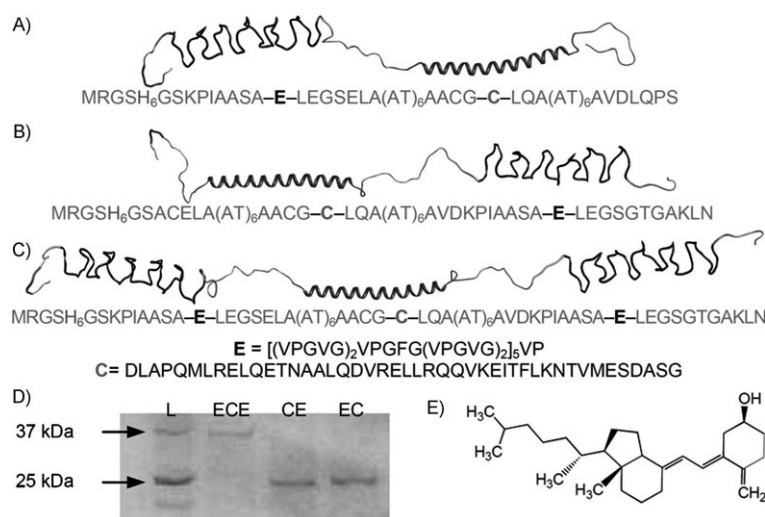

Figure 1. Amino acid sequences and structures of A) EC, B) CE and C) ECE. D) SDS-PAGE identifying protein fusions. E) Vitamin D₃.

tion; E undergoes phase separation[8] while C can bind small molecules.[9] Each polymer consists of compositionally identical E and C motifs into which a short $A_2(TA)_n$ spacer is incorporated at the juncture between the domains to ensure that each block is able to self-assemble as required (Figure 1 A–C). A critical feature of smart biomaterials is the ability of the polymers to self-assemble as a function of environmental cues such as pH and ionic strength.[4–8] Previous studies have shown that elastin and coiled-coil domains can be influenced by pH and salt conditions.[5–8,10,11] The synthetic versatility of these block polymer constructs permits the exploration of how the orientation and the number of blocks influence their physicochemical properties.

All block polymers have been overexpressed, purified and characterised.[12] The molar masses of EC, CE and ECE are 22 731, 22 911 and 35 188 Da, respectively.[12] Although SDS-PAGE analysis of the purified polymers reveals a slightly higher molecular weight for EC, CE and ECE, due to the E portion of the block polymers (Figure 1 D), the exact masses were confirmed by MALDI.[12] To determine the conformations of the block polymers, far-UV circular dichroism (CD) measurements were conducted. The homopolymer C adopted a helical structure that exhibited a transition to random configuration as the temperature was raised. In contrast, E adopted an initial β-turn that loses its structure at higher temperatures.[12] Although nearly identical in composition, the EC and CE diblocks differed in secondary structure and exhibited distinct temperature-dependent conformational changes (Figure 2 A, B). In the case of

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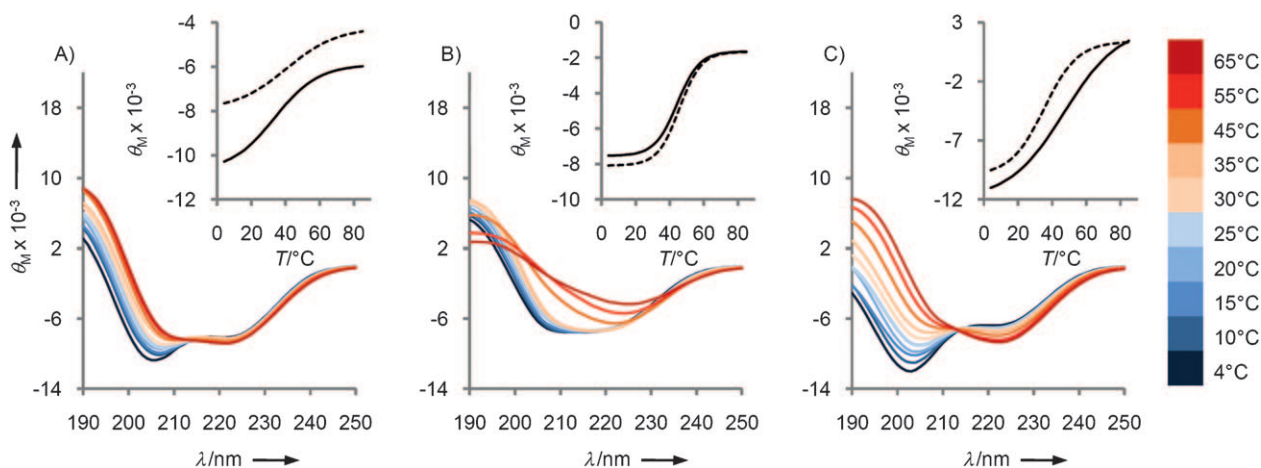
Figure 2. CD wavelength scans of A) EC, B) CE and C) ECE as a function of temperature. Insets represent the melting curves in the absence (solid line) and presence (broken line) of vD.

EC, a random-like structure was observed at low temperatures, which then transformed to a predominantly helical and β-conformation at higher temperatures, with a calculated melting temperature (T_m) of 33 °C (Figure 2 A).[12] The CE diblock revealed an overall random and β-structure at low temperatures that exhibited a transition to a red-shifted single minimum, indicative of a predominantly β-conformation with a T_m of 44 °C (Figure 2 B).[12] The ECE triblock showed a similar random-like conformation to the EC diblock at low temperatures. Upon an increase in temperature, ECE demonstrated a transition into a helix and finally to a β-conformation with a T_m of 47 °C (Figure 2 C).[12] The presence of the additional E domain not only increased the melting temperature, but also facilitated the formation of a β-conformation at high temperatures. Essentially, the overall polymer conformation is strongly influenced by the N-terminal fusion and the number of blocks.

Notably COMPcc can bind vitamin D_3 (vD; Figure 1 E) in the hydrophobic pore of the pentameric coiled-coil. In fact, the crystal structure demonstrates the binding of two vD molecules in the pore.[9] Moreover, CD analysis reveals that, for C in the presence of vD, a 6 °C increase in T_m is observed, while E with vD shows a 10 °C decrease in T_m.[12] The EC diblock incubated with vD shows a less random conformation at low temperatures with a $[\theta]_{222}/[\theta]_{208}$ of 0.83 relative to the unbound polymer of 0.79. An increase in T_m of 6.9 °C is observed for EC in the presence of vD; this indicates that the small molecule is binding to the polymer (Figure 2 A). The CE diblock incubated with vD demonstrates a slightly less random with a $[\theta]_{222}/[\theta]_{208}$ of 1.0, up from 0.96 when unbound; SELCON analysis reveals a β-structure upon an increase in temperature.[12] The polymer exhibits a modest enhancement in T_m by 1.3 °C in the presence of vD (Figure 2 B).

Like the EC diblock, the ECE triblock with vD reveals a less-random conformation with a $[\theta]_{222}/[\theta]_{208}$ of 0.72 relative to the unincubated polymer of 0.68.[12] The addition of vD to ECE results in a loss of the isosbestic point and a final structure at the highest temperature that is predominantly a β-conformation with a single minimum at 222 nm. This shift in conforma-

tion is shown by a large decrease in T_m of 10.7 °C (Figure 2 C), in contrast to the two diblocks.[12] In this case, the vD accelerates the conformational change from random to coil to β-structure and does so in a more cooperative fashion, as demonstrated by the highly sigmoidal curve, similar to that of the E homopolymer in the presence of vD.[12] For all three polymers, vD influences their microstructures as well as the overall conformation and melting points are altered, with an acceleration of β-conformation formation at elevated temperatures.

In addition to structural information, we have investigated the self-assembly of these block copolymers by dynamic light scattering (DLS, probing sizes of hundreds of nm or less, Figure 3) and small-angle light scattering (SALS, for sizes larger
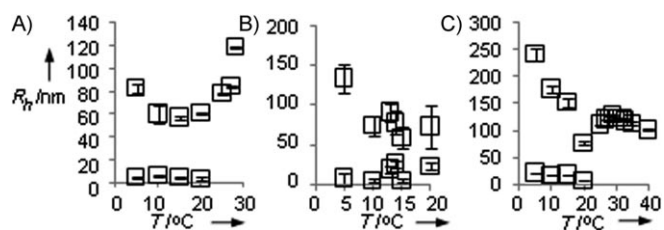

Figure 3. Hydrodynamic radius of A) EC, B) CE and C) ECE as a function of temperature derived from DLS.

than hundreds of nm).[12, 15] The C domains are known to form pentamers,[9] while the E assemble into β-spiral aggregates.[13, 14] EC shows two DLS modes below $T_t \sim 25$ °C and one mode above T_t. At lower temperatures, the two modes correspond to the unaggregated monomers (hydrodynamic radius, $R_h \sim 4$ nm) and aggregates with an average R_h decreasing from 85 to 60 nm with temperature. At higher temperatures, there is only one mode, which represents aggregates with R_h increasing with temperature (Figure 3 A). At even higher temperatures, the aggregates are too big to be determined by DLS. However, the SALS shows[12] that a characteristic length of

~21 µm is spontaneously selected and that the scattered intensity increases with time; this suggests spinodal decomposition.[15] The DLS behaviour of CE is qualitatively different from that of EC. There are more than two DLS modes,[12] although the sizes of aggregates corresponding to the first two modes are comparable (Figure 3B) to those of EC. The additional modes in DLS indicate that the aggregates are polydisperse with sizes larger than those measurable by DLS and increasing with time. This is further supported by SALS, where scattered intensity grows with time.[12] The ECE triblock exhibits a bimodal behaviour below $T_t \sim 25\,°C$, and only one aggregated structure above T_t (Figure 3C). The R_h of the aggregate (slow mode) decreases as T_t is approached. The R_h of the fast mode remains at ~20 nm below T_t. Above T_t, there is only one mode with an R_h of 125 nm. Unlike for the two diblocks, SALS experiments do not show any micron-scale features at temperatures above T_t. Thus, the overall macroscopic assembly of the polymer is dependent on the block orientation and number.

One of the central challenges in materials science is the ability to tailor functional "smart materials" that are capable of sensing and responding to specific environmental conditions.[16] These three protein block polymers have at least two distinct domains in which self-assembly can be triggered by temperature and small molecules. Although two of the polymers are compositionally similar, their physicochemical behaviour differs dramatically, thus suggesting that the orientation of the blocks plays a significant role—in stark contrast to synthetic block polymers. Moreover, the number of blocks contributes to the mode of assembly on both the micro- and macroscopic scale. The results provide insight into the design of smart protein polymers capable of self-assembly and storage of small molecules. Studies into the length dependence of the domains for assembly and storage of small molecules are underway.

Experimental Section

Detailed experimental protocols can be found in the Supporting Information.

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