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Self-assembled elastin-like polypeptide particles

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Abstract

In this work, the self-assembly of a recombinant elastin-based block copolymer containing both hydrophobic and cross-linking domains from the human elastin protein was investigated. The particle formation and dynamic behavior were characterized using inverted microscopy and dynamic light scattering. The morphology and stability were evaluated using scanning and transmission electron microscopy. Above a critical temperature the molecules self-assembled into a bimodal distribution of nano- and micron-sized particles. The larger particles increased in size through coalescence. Micron-sized particle formation appeared largely reversible, although a self-assembly/disassembly hysteresis was observed. At high polyethylene glycol (PEG) concentrations particle coalescence and settling were reduced, particle stability seemed enhanced and PEG coated the particles. Particle stabilization was also achieved through covalent cross-linking using glutaraldehyde. This study laid the foundation for optimization of particle size and stability through modification of the solvent system and has shown that this family of elastin-based polypeptides holds potential for use as particulate drug carriers.

Keywords: Elastin; Block copolymer; Self-assembly; PEG; Microparticles; Nanoparticles

1. Introduction

The complexity of nano- and microparticulate structures has been greatly expanded with the use of amphiphilic block copolymers that can microphase separate and self-assemble into higher-ordered structures. There is an increasing interest in using these structures as drug carrier systems [1–4], as polymer blocks can be used to control carrier surface properties and achieve compatibility between drug and block segments. Recently, water–soluble block copolymers have been designed with amphiphilicity, which can drive self-assembly through changes in solvent temperature, pH and electrolyte concentration [5,6]. In contrast to conventional block copolymers, this stimuli-responsive behavior can allow for mild aqueous self-assembly conditions, as well as triggered changes in structure size and shape.

The use of biologically based blocks is of great interest, particularly genetically engineered molecules. These molecules have a well-defined composition, monodisperse molecular weight, potential biocompatibility and are produced from a renewable resource. The advantages of genetically synthesized molecules make them suitable for pharmaceutical applications in which precise control over carrier systems and carrier material biocompatibility are required. Recombinant polypeptides, which incorporate functional sequences of the human elastin protein, have received considerable attention as they are amphiphilic block copolymers with alternating hydrophobic blocks and cross-linking domains [7–9]. The hydrophobic domains of the elastin protein are rich in valine (V), proline (P), alanine (A) and glycine (G) [10], and are often present in tetrapeptide, pentapeptide and hexapeptide tandem repeats, VPGG, VPGVG and VAPGVG, respectively, [9,11]. The hydrophobic domains facilitate both self-aggregation and elastomeric functions [12]. The cross-linking domains

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contain lysine residues (K), flanked either by alanine (A) or proline (P), and impart the ability to undergo covalent cross-linking. The groups of Woodhouse and Keeley have employed bacterial expression to synthesize a family of elastin-based polymers composed of elastin exon sequences, mimicking the native alternating structure of tropoelastin [13,14]. These polypeptides were designed to create a smaller, more versatile sequence while maintaining the functions of tropoelastin.

Elastin-based polypeptides are reported to phase separate from solution into microparticle structures through a coacervation process [12,18–23]. Upon further incubation of these microparticles, Bellingham et al. observed the formation of filamentous structures which are similar to fibers observed after assembly of native tropoelastin [13]. Similar fiber structures have been seen for other elastin-like polypeptide sequences [13,15–17]. The particulate structure could, therefore, be a precursor to the highly organized filamentous and fiber-like morphologies formed *in vitro*.

Although the self-assembled particles are thermodynamically stabilized through non-covalent interactions, the formation of these structures is generally reversible as their self-assembly is driven by factors which can be readily altered, including polymer concentration, solvent composition, solution temperature. Particle aggregation and structure reversibility, therefore, remain obstacles that must be overcome to form stable, isolated elastin-based block copolymer particles. Considerable attention has focused on strategies aimed to irreversibly stabilize the structures; one of which involves chemical cross-linking [18,24]. In a recent study, Clarke et al. provided evidence that full length tropoelastin formed coacervate particles in solution and the particle morphology could be stabilized through covalent cross-linking [25].

In the current study, we explore the assembly of EP20-24[4], an elastin-based block copolymer designed in collaboration with the Keeley research group, into elastin-based particles. The EP20-24[4] molecule was specifically chosen for the high number of hydrophobic domains in the sequence, which may enhance self-assembly in a similar hierarchical manner to full-length tropoelastin *in vitro* [13,18]. EP20-24[4] has the ability to self-assemble into particles, and the coacervate particle morphology can be stabilized through covalent cross-linking. In addition, particle formation, size and stability can be controlled using the solvent system. This work also provides a basis for evaluating the suitability of these elastin-based particles as drug carriers by investigating the incorporation of biologically inert molecules into the elastin-based polymer system.

2. Materials and methods

2.1. Reagents

All chemical reagents were purchased form Sigma–Aldrich Canada Ltd., (Oakville) unless otherwise noted. Milli-*Q* water was obtained from Millipore ultrafiltration system (Millipore Corp.).

2.2. Elastin-like polypeptide production

2.2.1. Polypeptide expression and purification

EP20-24[4] corresponds to the recombinant elastin-like polypeptide (EP) with exons 20-21-23-24-21-23-24-21-23-24-21-23-24 found in the human aortic elastin gene (see Supporting information available online). The construct is a glutathione S-transferase fusion protein [14,26]. The exon sequences were ligated into pGEX-2T vectors (Amersham Pharmacia Biotech Inc.), which were subsequently used to transform BL21(DE3) pLysSb cells (Stratagene). Polypeptide expression and purification was conducted using previously described protocols [26].

2.2.2. Polypeptide characterization

Polypeptide molecular weights were confirmed by matrix-assisted laser desorption–time of flight mass spectrometry at the Proteomic and Mass Spectrometry Center (Medical Sciences, University of Toronto). Amino acid compositions were determined by amino acid analysis at the Advance Protein Technology Center (Hospital for Sick Children) using norleucine as an internal standard. Polypeptide purity and concentrations were calculated using the molar ratios of the amino acids.

2.3. Coacervation temperature characterization

A total sample volume of 1 ml was placed into a quartz cuvette, inserted into a UV–Vis spectrophotometer (Varian Cary 3 spectrophotometer, Varian Inc., Melbourne, Victoria, Australia) equipped with a temperature controller. The absorbance was measured at 400 nm during heating at a rate of 1 °C min^{-1} and during cooling at a rate of 1–5 °C min^{-1}. The coacervation temperature was determined as the temperature on the turbidity profile, which displayed the maximum slope during the absorbance increase.

2.4. Particle preparation

Solutions of EP20-24[4] were prepared at a concentration of 2 mg ml^{-1} in 1 M NaCl phosphate-buffered saline (PBS). PEG-2000 (MW = 1900–2200) stock solutions in PBS were prepared and added to the sample solutions to achieve 5–20 wt./vol.%. The solution was vortexed, and left on ice for a minimum of 1 h. Coacervation was induced through incubation at the specified temperatures under stagnant conditions. As required, a homobifunctional amine cross-linker, glutaraldehyde (8% in water, Fisher Scientific Ltd.), was added to a final concentration of 0.5 vol.% under stagnant conditions. Following desired incubation, the samples were maintained at 37 °C for 10 min, removed from heat and diluted with an equivalent volume of distilled water.

ID	Title	Pages
1969	Self-assembled elastin-like polypeptide particles	9