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Transient tropoelastin nanoparticles are early-stage intermediates in the coacervation of human tropoelastin whose aggregation is facilitated by heparan sulfate and heparin decasaccharides

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ABSTRACT

Tropoelastin assembly is a key step in the formation of elastin. We consider how nanoscale intracellular assemblies of tropoelastin can congregate in an extracellular environment to give microscale aggregates. We describe novel 200–300 nm spherical particles that serve as intermediates in the formation of the coacervate. Their aggregation gives 800 nm to 1 μm species. This process is facilitated by heparan sulfate and dermatan sulfate interactions which effectively lower the critical concentration to facilitate this transition. This coacervation process was examined using a panel of heparin chains of various lengths and showed greatest efficacy for the decasaccharide, followed by the octasaccharide, while the hexasaccharide displayed the shortest efficacious length. We propose that these oligosaccharide interactions enable the charge-mediated aggregation of positively charged tropoelastin. This biochemistry models glycosaminoglycan interactions on the cell surface during elastogenesis which is characterized by the clustering of nascent tropoelastin aggregates to form micron-sized spherules.

1. Introduction

Tropoelastin is the soluble 60–70 kDa precursor of elastin (Mithieux and Weiss, 2005). Tropoelastin monomers self-assemble though coacervation which aligns and concentrates the molecules (Urry et al., 1978). At low temperatures, tropoelastin is soluble in solution, because water forms a clathrate-like structure around the tropoelastin hydrophobic regions which keeps the molecule unfolded. On raising the temperature, the clathrate-like structure of water is disrupted, which exposes the hydrophobic domains and allows them to interact with other hydrophobic domains (Urry et al., 1969, 1974; Urry and Long, 1977). Optimal coacervation of human tropoelastin occurs at 37 °C, 150 mM NaCl with pH 7–8 (Vrhovski et al., 1997).

The early stages of tropoelastin assembly in elastogenesis are only partly understood. There is a disparity between the sizes of the intracellular ~300 nm vesicular aggregates that culminate in tropoelastin secretion (Hucthagowder et al., 2009) and the larger ~1 μm coacervate particles that are seen on the cell surface (Kozel et al., 2006). These interactions involve cell surface glycosaminoglycans, particularly heparan sulfate as modeled by heparin (Broekelmann et al., 2005; Cain et al., 2005; Gheduzzi et al., 2005). The elastogenic cell surface is blanketed by a negatively charged glycocalyx that is dominated by heparan sulfate (Perrimon and Bernfield, 2000). Charged glycosaminoglycans bind tropoelastin and mediate coacervation through charge contributions by the lysine side chains on the molecules (Wu et al., 1999). Glycosaminoglycans such as heparin enable the coacervation of synthetic human tropoelastin at concentrations substantially below the critical (i.e. threshold) concentration (Tu and Weiss, 2008). Indeed tropoelastin's remarkably high pI has a counterpart in heparin and heparan sulfate which are amongst the most negatively charged biomolecules that have been identified (Perrimon and Bernfield, 2000). Here we assess the capacity of heparan sulfate and heparin oligosaccharides to promote the aggregation of tropoelastin nanoaggregates to form micron-sized particles.

Tropoelastin coacervation can be distinguished into four distinct stages: ~1–2 μm protein spherules, larger ~6 μm droplet formation, fusion of droplets followed by the formation of a coalesced layer (Tu and Weiss, 2008). No intermediates have been detected in the progression from monomer tropoelastin (~15 nm) to these assembly stages (Clarke et al., 2005, 2006; Wise and Weiss, 2009). This means that there is a rapid monomer to *n*-mer assembly of monomers to reach the critical concentration which is above 1 mg/ml tropoelastin (Toonkool et al., 2001).

We show here that as a biochemical model of the elastogenic cell surface, heparan sulfate promotes the stabilization and aggregation of ~200 nm tropoelastin nanoparticles. Using a panel of heparin oligosaccharides, we show that the most effective glycosaminoglycan length is a decasaccharide.

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2. Results

2.1. Heparin derived oligosaccharides can promote tropoelastin coacervation

A sub-critical concentration of 120 μg/ml tropoelastin in PBS was selected because it is one-tenth of the minimum amount required for coacervation in the absence of glycosaminoglycans (Wu et al., 1999; Toonkool et al., 2001). Various lengths of heparin-derived oligosaccharides were used to assess the efficacy of the coacervation of this solution at 37 °C (Tu and Weiss, 2008) where each oligosaccharide was kept at a constant concentration of 20 μg/ml. The 10-mer and full-length heparin promoted coacervation to a similar extent. The 8-mer enabled some coacervation, while negligible coacervation occurred in the presence of 6-mer, 4-mer and 2-mer (Fig. 1).

To assess the lowest effective concentration for each length of oligosaccharide, their various concentrations were tested for their ability to detectably coacervate tropoelastin that was maintained at 120 μg/ml in PBS. The 10-mer facilitated coacervation at 5 μg/ml oligosaccharide (Fig. 2A). The 8-mer required at least 20 μg/ml oligosaccharide, where 50 μg/ml gave an effect comparable to 20 μg/ml 10-mer (Fig. 2B). Tropoelastin started to coacervate only if the concentration of 6-mer was at least 100 μg/ml (Fig. 2C). Up to 200 μg/ml of the 4-mer and 2-mer showed no effect on coacervation.

The critical concentrations for coacervation of tropoelastin were assessed in the presence of 20 μg/ml heparin oligosaccharides. At least 30 μg/ml tropoelastin was needed to achieve coacervation with the 10-mer, 60 μg/ml tropoelastin for the 8-mer.

2.2. Tropoelastin coacervation is modulated by heparin derived oligosaccharides

Full-length, 10-mer, 8-mer and 6-mer heparin were prepared in PBS at 20 μg/ml and 120 μg/ml tropoelastin was added to these solutions. Turbidity at 300 nm was assessed after 10 min at various temperatures from 10 to 70 °C, then the maximum change of absorbance at each temperature was recorded and used to express a percentage of the maximum turbidity for each sample, in order to generate a series of coacervation curves. The midpoints of coacervation were approximately 17 °C, 32 °C, 37 °C, 40 °C and 46 °C for heparin, 10-mer, 8-mer, 6-mer and the no-heparin control, respectively (Fig. 3). The 4-mer and 2-mer showed the same result as the negative control. Of the oligosaccharides, only the 10-mer was capable of achieving at least 90% coacervation at 37 °C.

2.3. Nanoparticle formation during coacervation is modulated by oligosaccharide length

Tropoelastin at 120 μg/ml in PBS was a monodisperse ~15 nm monomer population at 20 °C and 25 °C (Toonkool et al., 2001; Clarke

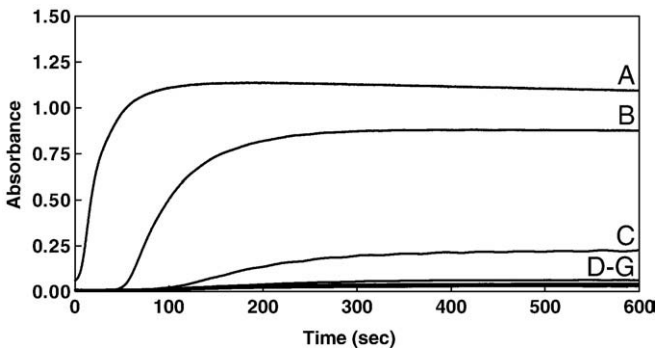

Fig. 1. Coacervation of 120 μg/ml tropoelastin at 37 °C in the presence of 20 μg/ml of (A) heparin, (B) heparin 10-mer, (C) heparin 8-mer, (D) heparin 6-mer, (E) heparin 4-mer, (F) heparin 2-mer and (G) in the absence of heparin.

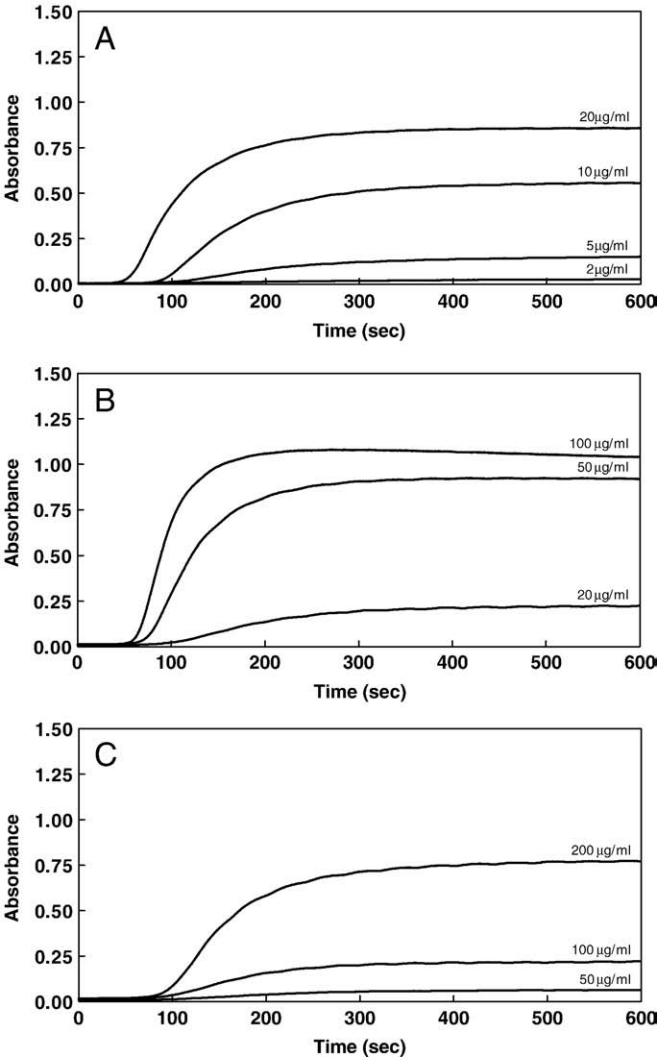

Fig. 2. Coacervation of 120 μg/ml tropoelastin at 37 °C in the presence of (A) 10-mer, (B) 8-mer and (C) 6-mer heparin.

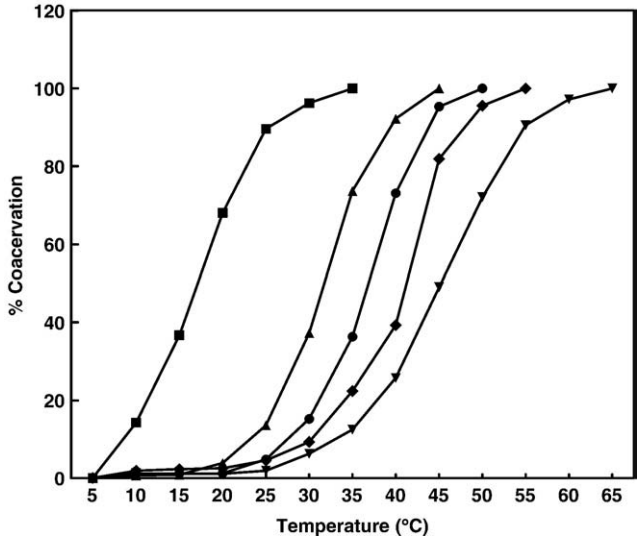

Fig. 3. Tropoelastin (120 μg/ml) coacervation in the presence of 20 μg/ml of heparin (■), 10-mer (▲), 8-mer (●), 6-mer (♦), and in the absence of heparin (▼).