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Effect of peptide self-assembly on the rheological properties of alginate-peptide conjugates solutions

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ABSTRACT

Alginate, a polysaccharide that gels in the presence of divalent ions is an obvious component of an injectable gel for regenerative medicine. Covalent bonding peptides to alginates is routinely used tailor alginate's biofunctionality. Here, we present a systematic investigation of the effect of three arginine-glycine-aspartic acid (RGD) -containing peptides, G_6KRGDY, A_6KRGDY and V_6KRGDY, on the mechanical properties and spatial organization of an alginate-peptides hybrids. Small angle X-ray Scattering (SAXS) and rheology measurements and analysis showed that the peptide sequence and its ability to self-assemble in aqueous solution is an important factor in defining the properties of the alginate-peptide hybrids. While the dominant factor in determining the properties of Alginate-G_6KRGDY is the electrostatic interactions between the peptide and polymer; For Alginate-A_6KRGDY and Alginate-V_6KRGDY the ability to form H-bonds is their most significant trade. Therefore, possible intermolecular interactions between the peptides and the polymer should be taken into consideration in the design of hybrid biomaterials.

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1. Introduction

Alginates are linear polysaccharides composed of 1 → 4 linked β-d-mannuronic acid (M) and its C-5 epimer, α-l-guluronic acid (G) produced by brown algae or bacteria [1,2]. They are block co-polymers, composed of widely varying M-block and G-block residues interspersed with MG sequences (MG-blocks). The amount and distribution of each monomer depends on the species and location of seaweed or bacteria from which the alginate is isolated. Addition of divalent ions (e.g. calcium ions) to alginate solution have been shown to induce alginate chain-chain association that results in the formation of an alginate gel [3]. Due to their natural origin, biocompatibility, biodegradability and facile gelation, alginates have been utilized for various applications in the food, textile and pharmaceutical industries both in solution and gel form [4–6].

Injectable hydrogels formed by in situ chemical polymerization or by the sol–gel phase transition have recently been paid much attention for biomedical applications such as regenerative medicine [7–9]. These material systems are viscous aqueous solutions before administration, but once injected, rapidly gel under physiological conditions. These hydrogels are appealing due to their potential to replace open surgery with a minimally invasive procedures [10,11]. The success of any injectable approach lies mainly in the gelation process, along with the physical, chemical, and biological properties of the hydrogel. The viscosity of the gel's components (i.e. the viscosity of the polymer's solution) is an important factor in the design of injectable gels since it will not only determine ease of administration but also the dispersion of incorporated small molecules such as drugs and the mechanical properties of the resulting gel [12].

The conformation of solvated polyelectrolytes and the microstructure of their solutions may be rationalized using scaling theories [13] that outline the solution behavior of polyelectrolytes as a function of their concentration. For polyelectrolytes the semi-dilute regime can be divided into two sub regimes, the un-entangled and entangled regimes [14]. Wyatt et al. [15] measured the viscosity as a function of concentration for the polysaccharide xanthan gum in both salt-free solution and in low salt concentration and compared the results with a scaling theory for polyelectrolytes. In salt-free solution, four concentration regimes of viscosity scaling and three associated critical concentrations were observed (c*, ce and cD) while in the salt solution, there were only three concentration

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regimes and two critical concentrations were observed (c* and ce). The observed viscosity-concentration scaling was in very good agreement with theory in the semi-dilute unentangled and semi-dilute entangled regimes in solution [14], demonstrating the ability to use scaling theories to describe the behavior of charged polysaccharides. Addition of salt to a charged polysaccharide solutions in the semi-dilute regime was shown to result in two competing effects: a decrease in viscosity due to charge screening, and an increase in viscosity due to H-bond formation [16,17].

Over the years Small angle X-ray scattering (SAXS) has been widely utilized for investigating different aspects of biopolymers in solution [18,19] including alginate [17,20,21]. SAXS patterns of charged polymers in a salt-free solution exhibit a characteristic correlation peak with a maximum q_0, corresponding to an average distance, $d_0 = 2\pi/q_0$, which represents the average distance between the charged domains [22,23]. Wang et al. [20] explored the effect of polymer concentration and of added salt on the structural features of alginate in aqueous solutions. Their findings show that the scattering maximum shifts toward the higher angle region with increasing alginate concentration and disappears in excess of added simple salt (i.e. monovalent ions). Recently we have shown that a scattering patterns of alginate in phosphate buffered saline (PBS), a salt solution of phosphate, KCl and NaCl (pH = 7.4), also do not exhibit the characteristic correlation peak [17].

Being a two component physical gelling system, alginate gained a lot of attention as potential in-situ gels for tissue engineering and regenerative medicine [6,24]. However, in spite of its many advantages, alginates suffer from several shortcomings including lack of ability to form specific cellular interactions. One of the approaches to overcome these drawbacks is to modify the alginate via chemical and physical reactions to obtain derivatives with various structures and properties [25–27]. Tuning alginate's properties such as biodegradability and cell affinity has been achieved through immobilization of specific ligands such as peptides and sugar molecules [28,29].Usually, the amino acid sequence of these peptides is chosen based on its desired function. For example the peptide sequence arginine-glycine-aspartic acid (RGD) have been routinely covalently attached to alginate to promote cell adhesion through integrin receptor in hydrogel scaffolds [28,30].

Peptides have also been explored as useful building blocks for the construction of various self-assembled nanostructures, such as, spheres, tubes, cylinders [31,32]. β-sheet-forming peptides have gained much interest due to their capability to form one dimensional (1D) nanostructures and 3D networks through intermolecular hydrogen bonding [33,34]. A peptide propensity to form β-sheets was also utilized by Elder et al. [35] to alter the viscosity of HA by covalently binding the $(LS)_4$ peptide to the HA backbone. As a result, in the dilute regime the modified HA exhibits significant increases in low-shear viscosities in comparison with the unmodified HA. Attaching alanine (lower propensity for β-sheet) [36] to HA did not lead to a significant change in the HA viscosity.

Typically, in the design of a covalently bound polymer-peptide hybrid for regenerative medicine the peptide's most important parameter is its bio-functionality and less attention is paid to the non-bioactive segment of the peptide sequence. However, the peptide ability to form non-covalent interactions with both the polymer and other peptides may affect the polymer-peptide physical properties, which in turn can influence its applicability as an injectable gel. Thus a fundamental understanding of the solution rheology of polymer-peptide conjugates is essential.

In this study, we undertook a systematic investigation of the effect of three covalently linked RGD-containing peptides, G_6KRGDY, A_6KRGDY and V_6KRGDY (at ratio of 1:100 mg/mg), on the physical properties of alginate in aqueous solutions. Our findings suggest that the peptide ability to self-assemble in aqueous solution is a significant factor in determining the viscosity of conjugated alginate-peptide aqueous solutions.

2. Materials and methods

2.1. Materials

Alginate (HF420RBS 70% guluronate, sodium salt, $\overline{Mw}$ = 475 kDa) was a kind gift from FMC Biopolymers (Dramen, Norway). NaOH, 2-[*N*-morpholio]ethanesulfonic acid (MES-buffer)], *N*-hydroxysulfosuccinimide sodium salt (sulfo-NHS), and *N*-(3-dimetylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC) were purchased from Sigma-Aldrich. The peptides A_6KRGDY and V_6KRGDY (90% grade) were purchased from Bio-Site/American Peptide Company (Vista, CA). G_6KRGDY (90% grade) was purchased from GenScript (Piscataway, New Jersey).

2.2. Peptide-polymer conjugation

Conjugation of a peptide to the alginate was performed using carbodiimide chemistry, according to Rowley et al. [37,38] Alginate was dissolved in the stock MES buffer (0.1 M) to give a 1% w/v aqueous solution. The pH was adjusted to 7.0 by adding NaOH (0.5 M), followed by stirring for 12–20 h. Sulfo-NHS was added in an amount that would give a ratio of sulfo-NHS:EDC of 1:2. EDC was then added as 50% of uronic acids (COOH). The peptide was added after 5 min (1:100 mg/mg peptide:polymer), and the solution was then stirred continuously for 30 min. Thereafter, the solution was dialyzed against triply distilled water in 3500 MWCO dialysis tubes for 96 h and then lyophilized for 72 h. For analysis, the requisite amount of the dried conjugated polymer was dissolved in triply distilled water (18.2 mΩ/cm).

2.3. Small angle X-ray scattering (SAXS)

Small angle x-ray scattering patterns of polymer solutions were obtained with a SAXSLAB GANESHA 300-XL. CuK_α radiation was generated by a Genix 3D Cu-source with an integrated monochromator, 3-pinhole collimation and a two-dimensional Pilatus 300K detector. The scattering intensity q was recorded at intervals of $0.012 < q < 3$ Å^{-1} (corresponding to lengths of 10–800 Å). Measurements were performed under vacuum at the ambient temperature. The scattering curves were corrected for counting time and sample absorption. The solution under study was sealed in thin-walled quartz capillaries about 1.5 mm in diameter and 0.01 mm wall thickness.

SAXS experiments were also performed on the BM29 beamline at the European Synchrotron Radiation Facility (ESRF) in Grenoble, France. An energy of 12.5 keV corresponding to a wavelength of 0.998 Å was selected. The scattering intensity was recorded using a Pilatus 1M detector, in the interval $0.004 < q < 0.5$ Å^{-1} (corresponding to lengths of 50–1500 Å). Ten frames with 2-s exposure time were recorded for each sample. Measurements were performed in flow mode, in which samples are pushed through the capillary at a constant flow rate. The dedicated beamline software BsxCuBe was used for data collection and processing. The scattering spectra of the solvent were subtracted from the corresponding solution data using the Irena package for analysis of small-angle scattering data [39]. Data analysis was based on fitting the scattering curve to an appropriate model by software provided by NIST (NIST SANS analysis version 7.0 on IGOR) [40] and Model Plot [21].

2.4. Model fitting of small-angle scattering patterns

The scattering of a spherical object with uniform electron

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