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Mechanisms of oligoguluronate modulating the calcium-induced gelation of alginate

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ABSTRACT

The modulatory effect of oligoguluronate (GB) on the gelation of macromolecular alginate (ALG) was investigated by means of dynamic oscillatory rheology, fluorescence and AFM measurements. An inhibitory effect in the range of $0.25 < R$ (Ca/G) < 0.60 and a promotive effect in the range of $R > 0.60$, were quantified. The two effects are thought to be associated with the different molecular events that dominate the gelation of alginate, namely, egg-box dimerization and lateral aggregation. Quantitative analysis indicates a competitive binding rather than a screening binding during egg-box dimerization, which is responsible for the inhibitory effect in the lower Ca concentration regime. On the other hand, in the higher Ca concentration regime where alginate gelation is predominated by chain lateral aggregation, the dimers formed by GB could act as a binder to enhance the aggregation of alginate dimers, resulting in a promotive effect on alginate gelation. The results are consistent with the microstructures observed by AFM.

1. Introduction

Alginate is a family of unbranched binary copolymers of (1 → 4)-β-linked-D-mannuronic acid (M) and α-L-guluronic acid (G) of widely varying composition and sequence [1]. It is composed of homopolymeric regions of M and G residues, interspersed with regions of alternating structure MG [2]. These regions are termed as M-, G-, and MG-blocks, respectively. Multivalent cations, such as Ca^{2+}, Sr^{2+}, Ba^{2+}, Fe^{3+} and Al^{3+}, can induce the gelation of alginate aqueous system by mediating chain association [3]. The gelation mechanism has been described by the famous "egg-box" model proposed by Rees et al., in which divalent cations particularly Ca ions are preferentially chelated by a pair of oppositely facing G sequences under specific coordination interactions to form egg-box dimers [4,5]. In the presence of sufficient Ca^{2+}, the dimers can further grow into multimers by lateral association [6–9]. Fiber X-ray diffraction suggested that the lateral association between dimers takes place through non-specific interactions such as water-mediated hydrogen bonding and disordered Na^{+} and Ca^{2+} cations [10]. This is because the nonzero intensity of the 001 diffraction signal excludes the possibility of long-range crystallographic packing between dimers. Based on stepwise titration of Ca into dilute alginate solution, we proposed a multiple-step binding of Ca to alginate [11]. The dimerization process was found to be highly critical, only occurring when the stoichiometry of egg-box dimers is met, that is, Ca/G = 0.25. Prior to the dimerization, mono-complexation involving Ca ions condensed on to a single alginate chain took place. The lateral association of dimers into multimers was less critical, occurring at Ca/G ≈ 0.55, which is slightly higher than the stoichiometry of egg-box multimers (0.50). The reason is presumably due to the unspecific nature of the interactions that drive the lateral association. A further study on concentrated solutions showed that the gelation and gel properties of alginate were

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influenced by Ca concentration regimes where different binding steps dominate [12]. Borgogna and the coworkers recently proposed a tilted egg-box model for the initial binding of Ca to alginate, in contrast to the classical cooperative egg-box arrangement [13]. The tilted egg-box structure should be conceived of as loosely bound and transient cross-links that hold two alginate chains together by a short-range electrostatic attraction under the mediation of Ca ions.

Most of the important applications of alginate in the food, pharmaceutical and (bio)-medical industries are connected with its Ca-binding and gel-forming abilities [14–18]. For this reason, precisely controlling and tailoring these properties become subjects of great technological importance. Intrinsic structural parameters associated with alginate, including M/G ratio, blockiness, and molecular weight, can be used to tune the calcium-binding and gelling properties of alginate [19,20]. Remarkably, the availability of epimerases in addition to lyase enabled the modification of fine chemical structure and molecular weight of alginate [21,22]. External parameters such as Ca concentration, alginate concentration, ionic strength, and ways of introducing Ca ions (e.g., internal gelation vs. external gelation) have also been employed to alter the gelation and gel properties of alginate [2].

It has been previously reported that oligoguluronate, namely guluronate block (GB) with a molecular weight of typically some thousands, can be used as modulators of gelation kinetics as well as local network structure formation and the equilibrium properties of alginate gels [3,23,24]. GB can be prepared from general alginate according to a patented procedure by taking advantage of the different susceptibility of M-, MG- and G-blocks towards acid hydrolysis and their different inherent acid solubility [25]. Possible molecular mechanisms have been suggested: 1) GB sequesters Ca in Ca-limited regime either by binding to oligoguluronate sequences of the existing gel network or between free oligoguluronates; 2) in Ca-excessive regime GB may act as binders between topologically restricted alginate chains to shorten the elastic segments of gel network [24]. The modulatory effects of GB have been exploited for potential food, pharmaceutical and medical applications. For example, GB can be used to replace phosphate in alginate-containing food products, since the intake of high level of phosphate may bring health related consequences [26]. GB was also found to be able to disrupt the intermolecular interactions in complex mucous systems and hence to modify mucus rheology. This led to an interesting application of GB as a pseudo-mucolytic agent to improve lung function in patients suffering from cystic fibrosis [27,28].

In these contexts, there is a need for a better understanding of the precise mechanisms of GB modulating the Ca-mediated interaction, structural organization, gelation and gel properties of macromolecular alginate. Here, gelation kinetics and equilibrium gel properties of alginate aqueous solutions induced by in-situ release of Ca ions from Ca-EDTA during D-glucono-δ-lactone (GDL) hydrolysis were measured and the modulatory effects of GB analyzed quantitatively. These together with fluorescence and AFM measurements led to a clearer picture depicting the modes of interactions between GB and alginate.

2. Materials and methods

2.1. Materials

Sodium type alginate (ALG) was kindly provided by FMC BioPolymer (Norway) and used without further purification. Disodium calcium salt of ethylenediaminetetraacetic ($CaNa_2$-EDTA·$2H_2O$) and D-glucono-δ-lactone (GDL) were purchased from Aladdin Chemistry (China). *N*-hydroxysuccinimide (NHS), 2-[*N*-morpholino]-ethane-sulfonic acid (MES), Rhodamine 123, and 1-ethyl-3-[3-(dimethylamino)propyl]-carbodiimide (EDC) were from Sigma–Aldrich (China). All the reagents were of analytical grade. Milli-Q water was used in all experiments.

2.2. Preparation of GB

Oligoguluronate, namely guluronate block (GB) was produced from ALG by means of acid hydrolysis according to a patented method with slight modification [25]. In brief, 20 g of ALG was added into 200 mL of 0.3 M HCl and agitated overnight, followed by hydrolysis at 95 °C for 5 h. The hydrolyzed solution was subjected to centrifuge at 750 g for 15 min to separate precipitates. The precipitates were re-suspended in 200 mL of 0.3 M HCl and washed three times by using the same centrifugal procedures. The washed precipitates were dissolved in 200 ml Milli-Q water and was adjusted to pH 3.5 using NaOH. After agitation overnight, the undissolved fraction was separated by centrifuge at 750 g for 15 min and washed three times using Milli-Q water (pH 3.5). The undissolved fraction was brought into dissolution by adjusting pH to 7.0 using NaOH, followed by filtration (0.45 μm pore size) and freeze-drying to obtain GB sample.

2.3. Structural and molecular characterization of alginate

Structural and molecular parameters of alginate samples, ALG and GB, were characterized by ^{1}H-nuclear magnetic resonance (^{1}H-NMR) and gel permeation chromatography-multiangle laser light scattering (GPC-MALLS). Prior to ^{1}H-NMR measurements, the high molecular weight alginate ALG was slightly degraded by mild hydrolysis (pH3.0, 90 °C for 30 min) to improve signal resolution. The pre-treated ALG and low molecular weight GB were dissolved in D_2O at a concentration of 20 mg/mL. EDTA (7.5 mg/mL) was added to prevent traces of divalent cations from interacting with the alginate samples. ^{1}H-NMR spectra were recorded on a 500 MHz Bruker UltraShield NMR spectrometer operated at 70 °C. 4,4-dimethyl-4-silapentane-1-sulfonic acid (DSS) was used as internal standard. Chemical composition (M/G content) and sequence information, specified in terms of the mean fraction of selected diads (i.e. MM, GG, MG) and triads (i.e. GGG, GGM, MGM), of the alginate samples were calculated from NMR spectra according to the method reported by Grasdalen et al. [29,30]. The average length of G-blocks larger than one $N_{G>1}$ was derived from the equation $N_{G>1} = (F_G - F_{MGM})/F_{GGM}$ where F_G and F_{MGM} stand for the fractions of G monomer and MGM triad, respectively.

GPC-MALLS was employed to determine the weight-average molar mass (M_w), radius of gyration (R_g), and polydispersity index (M_w/M_n) of the alginate samples. The system consisted in series of a Shodex OHpak SB-805HQ separating column, a DAWN HELEOS multiangle light scattering detector (Wyatt Technology Corporation, USA) operated at 658 nm, and an Optilab rEX refractometer (Wyatt Technology Corporation, USA). 0.2 M aqueous NaCl solution containing 0.005% NaN_3 was used as eluent and pumped at a constant rate of 0.45 ml/min. 200 μL of ALG and GB (0.2 mg/mL and 1 mg/mL, respectively) were injected into the GPC-MALLS system for analysis after filtration through 0.45 μm nylon filters. A dn/dc value of 0.150 mL/g was used for analysis.

2.4. Rheological measurements

Rheological measurements were used to characterize the gelation kinetics and equilibrium gel properties of alginate aqueous solutions induced by in-situ release of Ca^{2+} ions from Ca-EDTA