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Alginate esters via chemoselective carboxyl group modification

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ABSTRACT

Alginates are (1 → 4) linked linear copolysaccharides composed of β-D-mannuronic acid (M) and its C-5 epimer, α-L-guluronic acid (G). Several strategies for synthesis of carboxyl modified alginate derivatives exist in the literature. Most of these however employ aqueous chemistries, such as carbodiimide coupling reactions. Based on our recently discovered method for homogeneous dissolution of tetrabutylammonium (TBA)-alginate, we now describe use of tetrabutylammonium fluoride (TBAF)-based two component solvent systems as media for synthesis of carboxyl-modified alginate esters. Partially and fully esterified benzyl, butyl, ethyl, and methyl alginates were synthesized via reaction with the corresponding alkyl halides. The newly synthesized derivatives were soluble in polar aprotic solvents without the addition of TBAF. Saponification was performed to demonstrate that alkylation was completely regioselective for carboxylate groups in preference to hydroxyl groups to form esters. We demonstrate the utility of these alginate esters to enhance aqueous solubility of the flavonoid naringenin by formation of solid dispersions.

1. Introduction

Alginates are unbranched polysaccharides consisting of 1 → 4 linked β-D-mannuronic acid (M) and its C-5 epimer α-L-guluronic acid (G). They are isolated from one of two major sources – algae such as kelp, or as an exopolysaccharide of bacteria such as *Pseudomonas aeruginosa*. The alginate microstructure is unique and comprises of M-blocks and G-blocks interspersed with MG sequences (MG-blocks). Source and species dependent variability exists between alginates in terms of their copolymer composition, sequence and molecular weights. This diversity is often used as a tool to control final properties in a number of natural functions. For example, in *Laminaria hyperborea*, the stipe and holdfast require high strength and rigidity and therefore contain alginates having higher fractions of G residues. In contrast, the leaves of the plant which require flexibility for flotation in streaming water possess alginates with lower fractions of G residues. Due to the abundance of algae in water bodies, there is a large amount of alginate material present in nature. Industrial alginate production is approximately 30,000 metric tons annually, and is estimated to comprise less than 10% of the biosynthesized alginate material (Draget, 2009). There exists considerable additional potential to design sustainable biomaterials based on alginates.

One of the most obvious and effective ways to design high performance biomaterials is by chemically reacting the functional groups available on the alginate backbone. Alginate being a water soluble polysaccharide in salt form, the use of aqueous chemistries such as 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide/N-hydrosuccinimde (EDC/NHS) coupling reactions to make esters/amides is a widely used strategy (Pawar & Edgar, 2012). While aqueous phase reactions can be a good way to modify polysaccharides, they do not match the versatility that organic phase reactions can offer. This is clearly demonstrated through the rich body of literature published on organic dissolution and derivatization of cellulose (Liebert, Heinze, & Edgar, 2010). The fact that the body of literature on alginate derivatization is much smaller is due in part to the presence of ionizable carboxylic acid functionalities on the alginate backbone. These charges play a crucial role in dictating alginate organic solubility; in fact, until recently no organic solvent systems had been described that would permit full dissolution of alginate. Addressing this key problem, we have recently shown that it is possible to dissolve alginates in two-component organic media consisting of polar aprotic solvents (such as DMSO, DMF, DMAc or DMI) and the salt tetrabutylammonium fluoride (TBAF) (Pawar & Edgar, 2011). Water soluble alginate acetates up to DS_{acetyl} ~1.0 were synthesized by reaction of alginate hydroxyl groups in these solvent systems.

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A disadvantage with the TBAF based two-component solvent systems is that the salt is hydrated and there is always a small percentage of water present in the mixture. Furthermore, it has been shown by our group that TBAF is useful as a reagent for regioselective deacylation of cellulose esters (Xu & Edgar, 2012). Although not yet experimentally confirmed, a similar deacylation reaction occurring in situ could be the source of the limitation we have observed in alginate acylations, where alginate acetate product DS_{acetyl} has not exceeded ~1.0. We would therefore like to develop alginate derivatives that would dissolve in organic media without the addition of TBAF. From our past studies, no single component organic solvent (including ionic liquids) is capable of fully dissolving tetrabutylammonium (TBA) salts of alginic acid. The ability to dissolve alginates in water-free organic media would be advantageous in two ways. Firstly, it would permit the use of organic reagents that are incompatible with water, thereby enabling new pathways to alginate functionalization. Secondly, it would enable solvent-based processing methods, which may extend the applicability of alginates beyond the current scope (which involves mostly aqueous processing).

Substitution of alginate at the carboxyl group to form an ester is one potential approach to the preparation of derivatives with such properties. We are aware of two prior instances where alginate esters have been synthesized via carboxyl group modification, neither of which involves complete dissolution of alginate in organic solvents (Pawar & Edgar, 2012). The first derivative is a commercially available alginate ester, propylene glycol alginate, synthesized by reaction with propylene oxide. The second set of reports has detailed the reaction of TBA-alginate with dodecyl bromide to prepare dodecyl alginate in DMSO (Babak, Skotnikova, Lukina, Pelletier, Hubert, & Dellacherie, 2000; Leonard, Rastello de Boisseson, Hubert, & Dellacherie, 2004; Pelletier, Hubert, Lapicque, Payan, & Dellacherie, 2000). However, the maximum $DS_{dodecyl}$ value reported was 0.12. Our hypothesis was that by dissolving TBA-alginate homogeneously in TBAF containing solvent systems, and by using more reactive halide reagents, complete substitution (DS_{ester} = 1.0) would be possible, and intermediate degrees of substitution might be achievable by control of stoichiometry. Herein, we describe the use of bimolecular substitution nucleophilic (S_N2) reactions to esterify the alginate carboxylate groups, in order to seek access to fully as well as partially substituted derivatives. The solubility properties of the derivatives were studied and peak assignments were performed using alginates enriched in M and G residues.

One reason for our interest in more organic-soluble alginate carboxylate esters was our hypothesis that these amphiphilic polysaccharide derivatives might be useful as matrix polymers for amorphous solid dispersion (ASD) of drugs. Being able to dissolve alginates in organic solvents without the need for adding TBAF is a key processing step for the formation of ASDs. Therefore we report as part of this study the exploration of some of the alginate derivatives synthesized as amorphous solid dispersion polymers for the bioactive, poorly soluble, and poorly soluble flavonoid, naringenin (Nar).

2. Experimental

A. Materials: Four alginates were used for the study. We represent each as 'Mxxx', where M stands for mannuronate and xxx denotes the % of M residues in the sample. For example, M063 signifies a sample containing 63% M and 37% G residues. M100 alginate was isolated from an epimerase-negative mutant of *Pseudomonas fluorescens* (Chitnis & Ohman, 1990). M063 alginate, an alginic acid sodium salt from brown algae (low viscosity, Na-alginate) was from Sigma. M060 alginate was from FMC BioPolymer. M000 was an alginate containing G-blocks with an average $DP_n \sim 20$.

Water used in the experiments was deionized. Reagent alcohol (histological grade), hexanes (HPLC grade), DMSO (HPLC grade), DMF (HPLC grade), 5 N standardized NaOH solution, 1 N standardized HCl solution, NaI, KCl, potassium phosphate monobasic [KH_2PO_4] and ethyl acetate were purchased from Fisher Scientific (Fair Lawn, NJ). Benzyl bromide (98%) [BnBr], iodoethane (98%) [EtI], 1-iodobutane (98%) [BuI], 1-methyl-2-pyrrolidone (99.5%, extra dry) [NMP] and tetrabutylammonium hydroxide (TBAOH; 40 wt%, 1.5 M solution in water) were obtained from Acros (Fair Lawn, NJ). Deuterium oxide (99.9 atom% D; D_2O) containing 0.75% 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid sodium salt for NMR, (±)-naringenin, and iodomethane [MeI] were obtained from Sigma–Aldrich (St. Louis, MO). KBr used for FTIR analysis was purchased from International Crystal Labs (Garfield, NJ). DMSO-d_6 for NMR was acquired from Cambridge Isotope Laboratories, Inc. (Andover, MA).

B. Synthesis of TBA-alginate: For conversion of Na-alginate to its protonated form, a literature procedure (Babak, Skotnikova, Lukina, Pelletier, Hubert, & Dellacherie, 2000) was followed with modifications. HCl (0.6 N, 120 mL) was mixed with reagent alcohol (120 mL) in a beaker. Na-alginate (8 g) was added to the mixture and stirred overnight at 4 °C. The mixture was filtered, then the solid (alginic acid) was washed thoroughly, sequentially with alcohol and acetone. The solid product was dried overnight in vacuo at 60 °C. Fully dried alginic acid was then dispersed in 200 mL water. TBAOH was added dropwise with continuous stirring at room temperature until the polymer was dissolved and the pH was adjusted to 10.0. If the solution was too viscous, small amounts of water were added until a final viscosity was reached at which the solution could be easily stirred. The aqueous TBA-alginate solution was then premixed with reagent alcohol. The premix was precipitated in a mixture of ethyl acetate–hexane. The solvent ratios used were aqueous alginate acetate:ethanol:ethyl acetate:hexane = 1:3:12:3. The precipitate was then filtered under vacuum and dried in vacuo at 60 °C. The ^{1}H-NMR and FTIR spectra for TBA-alginate are shown in the Supplementary Material as Figs. S1 and S2 respectively.

^{1}H NMR (500 MHz, D_2O) δ 5.82–3.46 (m, alginate backbone), 3.19 (t, $NC\underline{H}_2CH_2CH_2CH_3$ of TBA), 1.67 (m, $NCH_2C\underline{H}_2CH_2CH_3$ of TBA), 1.40 (m, $NCH_2CH_2C\underline{H}_2CH_3$ of TBA), 0.97 (t, $NCH_2CH_2CH_2C\underline{H}_3$). FTIR (KBr pellet method): 3400 cm^{-1}, hydroxyl O—H stretching; 2850–2960 cm^{-1}, aliphatic C—H stretching, 1610 cm^{-1}, carboxylate C—O stretching. Elemental composition of TBA-alginate M060 measured by elemental analysis: C, 56.46%; H, 10.46%; N, 2.86%. This composition corresponds to roughly 80% TBA and 20% Na salt. Yield: 9.22 g; 67%.

C. Synthesis of benzyl alginate: TBAF (0.1 g, 0.32 mmol) was dissolved in DMF (10 mL) at room temperature. TBA-alginate (0.15 g) was then added and the mixture stirred until the polymer was dissolved. The temperature was then raised to 80 °C. Benzylation was carried out using either BnBr or benzyl iodide (BnI). In case of BnBr, the necessary equivalents of reagent were directly added to the alginate solution. For BnI, the reagent was synthesized starting from BnBr and then added to the alginate solution. Upon reagent addition, the solution was stirred at 80 °C for 30 min, after which it was added to ethyl acetate (~150 mL) to precipitate the product. The precipitate was recovered and dried in vacuo at 60 °C. Dry crude benzyl alginate was then dissolved in DMF (~1.5 mL), precipitated in ethyl acetate, filtered and dried in vacuo; this step was repeated one more time. The dry solid obtained thereafter was benzyl alginate. The synthesis of BnI reagent is described as follows.

Benzyl iodide: NaI (0.535 g, 3.6 mmol) was dissolved in 5 mL of acetone (dried over 3 Å molecular sieves) under N_2 at room temperature. To this solution BnBr (0.43 mL, 3.6 mmol) was added. Upon