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International Journal of Pharmaceutics 304 (2005) 40–50
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Inhibition of pepsin activity by alginates in vitro and the effect of epimerization

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Received 18 February 2005; received in revised form 16 July 2005; accepted 18 July 2005
Available online 1 September 2005

Abstract

Alginates are versatile biopolymers used extensively in the food, textile and pharmaceutical industries. One of the major uses is in the treatment of reflux disease and here we investigate whether alginates can influence pepsin activity, a major aggressor in reflux disease. The primary uronic acid structure of alginates can be altered using epimerase technology and we test tailor-made alginates to identify the optimal structure for pepsin inhibition. Pepsin activity in the presence of alginates was studied using an in vitro N-terminal assay and enzyme kinetics using a chromagenic peptide. The data described showed clearly that alginates were capable of concentration dependently reducing the activity of pepsin in a non-competitive manner, in vitro. This was variable between different alginates of wide ranging structure and size with positive correlation with alternating sequences of mannuronic and guluronic acid. We hypothesize that alginates may have a more extensive role in the treatment of reflux disease by inhibiting pepsin, a damaging component of the refluxate.

Keywords: Alginate; Pepsin; Gastroesophageal reflux; Epimerase; Enzyme kinetics

1. Introduction

Alginates are polysaccharides that provide the main structural component of brown algae (*Phaeophycae*) such as *Laminaria hyperborea*, *Lessonia nigrescens* and *Ascophyllum nodosum*. They are also produced as an exopolysaccharide by some bacteria such as *Pseudomonas aeruginosa* and *Azotobacter vinelandii*. Alginates are linear copolymers of (1–4) linked β-D-

Abbreviations: M, mannuronic acid; G, guluronic acid; GERD, gastro-esophageal reflux disease; ^{1}H NMR, nuclear magnetic resonance; *F*, molar fraction; SEC-MALLS, size exclusion chromatography-multi angle laser light scattering; TNBS, trinitrobenzenesulphonic acid; [*S*], substrate concentration; *V*, initial reaction velocity; V_{max}, maximum enzyme velocity; K_m, Michaelis–Menton constant; *r*, Pearson's correlation coefficient

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doi:10.1016/j.ijpharm.2005.07.017

mannuronic acid (M) and its C-5 epimer α-L-guluronic acid (G). The M and G residues can exist in homopolymeric regions (M block or G block) or heteropolymeric regions (MG block) (Smidsrod and Draget, 1996).

Alginates are among the most versatile biopolymers with respect to their uses in the food, textile and pharmaceutical industries (Onsoyen, 1996). They function as thickeners, stabilizers, gel-forming and film-forming agents. Within the pharmaceutical industry, applications include controlled release mediators, dental impression materials, wound dressings, anti-reflux medicines (Onsoyen, 1996) and microencapsulation (Skjak-Braek and Espevik, 1996). The many versatile applications of alginates are a function of their structure as different sequences of M and G infer different physical and chemical properties (Smidsrod and Draget, 1996).

It is now possible to control the primary structure of alginates and thereby the physical and chemical properties. The processes, genes and enzymes that control the structure of alginate produced by the bacteria *P. aeruginosa* and *A. vinelandii* are now well understood. The bacteria initially synthesize an alginate consisting solely of M residues (polymannuronate) and secrete it into the extracellular space. From this point the conversion of M into G within the polymer chain is catalyzed by mannuronan C-5 epimerase enzymes (May and Chakrabaty, 1994). The genes that encode the mannuronan C-5 epimerases have been isolated in both *P. aeruginosa* and *A. vinelandii* and designated $AlgE_1$–$AlgE_7$ each producing an enzyme with a different pattern of activity (Ertesvag et al., 1994; Svanem et al., 1999). They can then be used to modify the sequence of an alginate, either of seaweed or bacterial origin, in vitro (Ertesvag et al., 1994; Ertesvag et al., 1995). This enables the properties of alginates to be controlled and tailored to specific commercial applications.

Alginates have been demonstrated to possess several biological activities. Certain alginates, particularly those with high M content, have immune stimulating effects such as cytokine induction (Flo et al., 2000; Jahr et al., 1997) and protection against pathogenic invasion (Skjak-Braek and Espevik, 1996). Alginates exhibit bioadhesive ability to oesophageal tissue (Batchelor et al., 2002; Richardson et al., 2004, 2005) and can positively interact with mucin (Batchelor et al., 2000; Taylor et al., 2005). In preliminary studies, presented as meeting abstracts, alginates have been shown to upregulate the process of cell migration and so potentially speed up the repair of wounds (Dunne et al., 2002), protect rat gastric mucosa from stress and indomethacin induced ulcers (Del Buono et al., 2001). The process of fluid phase endocytosis is also upregulated in the presence of alginates (McPherson et al., 2002), they have the ability to reduce the activity of pepsin (Sunderland et al., 2000), and have intracellular molecular effects (Johnston et al., 2002; Dettmar et al., 2004).

Many of these bioactive properties have relevance to the treatment and/or prevention of gastro-esophageal reflux disease (GERD). This is the retrograde movement of the gastric contents into the esophagus resulting in symptoms of heartburn. Pepsin is a major aggressive factor in the gastric refluxate and is responsible for much of the esophageal damage seen in GERD sufferers (Salo et al., 1983; Tobey et al., 2001). Alginate containing products are currently used in the treatment of GERD (e.g. Gaviscon Advance®) although primarily as a physical barrier to prevent reflux. However, it is apparent that alginates may have greater potential for use in the treatment of GERD. Here we utilize epimerase technology to design new alginates and evaluate them for their ability to inhibit pepsin activity and to determine the alginate structure required for optimal activity in vitro with a long term view to pharmaceutical product development.

2. Materials and methods

2.1. Alginates

Current commercially available seaweed alginates were supplied by FMC Biopolymer, Drammen, Norway. Novel alginates of bacterial (mannuronan) and seaweed origin treated with the mannuronan C-5 epimerases $AlgE_1$, $AlgE_4$ and $AlgE_6$ were supplied by Gudmund Skjåk-Bræk, NOBIPOL, Trondheim, Norway. Alginates were characterized by ^{1}H NMR to resolve the fraction of monad, diad and triad frequencies (F_x) (Smidsrod and Draget, 1996), intrinsic viscosity and SEC-MALLS to determine molecular weight. Table 1 shows the 39 different alginates used in this