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The modulation of pancreatic lipase activity by alginates

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ABSTRACT

Alginates are comprised of mannuronic (M) and guluronic acid (G) and have been shown to inhibit enzyme activity. Pancreatic lipase is important in dietary triacylglycerol breakdown; reducing pancreatic lipase activity would reduce triacylglycerol breakdown resulting in lower amounts being absorbed by the body.

Lipase activity in the presence of biopolymers was assessed by enzymatic assay using natural and synthetic substrates. Alginate inhibited pancreatic lipase by a maximum of 72.2% (±4.1) with synthetic substrate (DGGR) and 58.0% (±9.7) with natural substrate. High-G alginates from *Laminaria hyperborea* seaweed inhibited pancreatic lipase to a significantly higher degree than High-M alginates from *Lessonia nigrescens*, showing that inhibition was related to alginate structure.

High-G alginates are effective inhibitors of pancreatic lipase and are used in the food industry at low levels. They could be included at higher levels in foods without altering organoleptic qualities, potentially reduce the uptake of dietary triacylglycerol aiding in weight management.

1. Introduction

In the western world, dietary fats can account for 40% of energy intake, with triacylglycerol (TAG) being the major component (Mu & Høy, 2004). Pancreatic lipase plays an important role in the hydrolysis of TAG with only 10–30% of hydrolysis occurring before the duodenal phase (Hamosh & Scow, 1973).

Pancreatic lipase has become a valid target in the treatment of obesity with the development of Tetrahydrolipstatin (orlistat®) (Drent & Vanderveen, 1993). Orlistat inhibits pancreatic lipase by covalently modifying the active site, reducing the hydrolysis of TAG (Borgstrom, 1988; Hadvary, Lengsfeld, & Wolfer, 1988). When taking orlistat, the level of ingested fat excreted in the faeces can be increased from 5% to 32% when compared to a placebo group (Zhi et al., 1994). In the UK, 98% of all prescriptions for the treatment of obesity in 2010 were for orlistat, the remaining 2% was for Sibutramine (withdrawn 2010) (The NHS Information Centre, 2012). Gastrointestinal side effects associated with orlistat treatment can often cause problems with patient compliance to the treatment regime, with below 50% compliance, even with pharmacist intervention (Gursoy, Erdogan, Cin, Cesur, & Baskal, 2006; Malone & Alger-Mayer, 2003). However, the gastrointestinal side effects of orlistat may be reduced if taken concomitantly with natural fibres, such as *Psyllium* mucilloid (Cavaliere, Floriano, & Medeiros-Neto, 2001).

Alginates are dietary fibres consisting of a linear polymer containing two epimers of uronic acid, mannuronic (M) and guluronic acid (G) (Haug & Smidsrod, 1967). Alginates can be extracted from the cell walls of brown seaweed or from certain bacteria. For example, alginates are the major constituents of the vegetative capsule of the rigid and desiccation resistant walls of metabolically dormant cysts in the soil bacteria *Azotobacter vinelandii* (Haug & Smidsrod, 1967).

Certain polymers have been shown to have an effect on triacylglycerol hydrolysis, such as chitin–chitosan mixtures and polydextrose with diethylaminoethyl groups attached (Han, Kimura, & Okuda, 1999; Tsujita et al., 2007). Both of these polymers potentially affect the substrate and the interface between substrate and enzyme.

Alginates have previously been shown to have an inhibitory effect on gastrointestinal enzymes. In 2000 Sunderland et al., showed that alginates reduced the activity of pepsin by an average of 52% *in vitro* (Sunderland, Dettmar, & Pearson, 2000). The work identified the characteristics of alginates that correlated with the level of pepsin inhibition (Sunderland, Dettmar, & Pearson, 2000). The molecular weight of the alginate was key to the level of pepsin inhibition achievable (Strugala, Kennington, Campbell, Skjak-Braek, & Dettmar, 2005; Sunderland et al., 2000).

The previously shown bioactivity of alginate can be altered by both sugar residue composition and molecular weight. The use of the epimerase enzymes allow alginates to be modified to a

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specifically desired ratio of M and G residues as well as the order of residues, therefore allowing designer alginates to be produced, which would be vital to the understanding of which characteristics of an alginate are important in a biological system.

Here we hypothesise that pancreatic lipase activity can be inhibited by alginates and that the extent can be modulated to a different degree dependent on the structural characteristics of alginate used. Well characterised alginates from both sources (bacteria and seaweed) were used in this study, including alginates that were enzymatically modified.

2. Materials and methods

2.1. Materials

All alginate samples were kindly provided by Technostics Limited (Hull, UK) (Table 1). The bile acids (deoxycholate sodium salt and taurodeoxycholate sodium salt) were both purchased from Fluka (Buchs, Switzerland). The lipase, colipase and orlistat (tetrahydrolipstatin), tris(hydroxymethyl)-methylamine, 1,2 Di-o-lauryl-rac-glycero-3-(glutaric acid 6-methyl resorufin ester) (DGGR), sodium acetate, calcium chloride and acetone were all purchased from Sigma–Aldrich (Poole, UK). The olive oil was purchased from a local supermarket (Cooperative Foods, UK) and the aluminium oxide was purchased from Fisher Scientific (Loughborough, UK).

2.2. Lipase activity assay using DGGR as the substrate

The lipase activity assay was a modified version of the method developed by Panteghini, Bonora, and Pagani (2001). The assay was comprised of three solutions; solution 1, solution 2 and the lipase solution. Solution 1; Tris buffer (50 mmol/l, pH 8.4 at 23 °C), 1 mg/l of colipase and 1.8 mM deoxycholate sodium salt. Solution 2; acetate buffer (18 mmol/l, pH 4.0 at 23 °C) 72 mM taurodeoxycholate sodium salt, 0.1 mM calcium chloride and 0.24 mM DGGR. Solution 2 was mixed with a magnetic stirrer at 500 rpm and 4 °C overnight. The lipase solution contains 1 g/l of porcine pancreatic lipase in deionised water, where 1 mg contains 60 U of lipase activity (where one unit will hydrolyse 1.0 microequivalent of fatty acid from a triglyceride in one hour at pH 7.4 using triacetin).

A 4 mg/ml stock solution of each polymer was prepared by slowly adding lyophilised biopolymer to the vortex formed by vigorously stirring solution 1 on a magnetic stirrer. The resulting stock solution (4 mg/ml) was then further diluted with solution 1 to achieve 1 and 0.25 mg/ml samples. This achieved a concentration of 3.43, 0.86 and 0.21 mg/ml, respectively in the reaction mixture. Two controls were used in the assay, an inhibition control (100% inhibition) and a lipase control (0% inhibition). The inhibition control contained 0.025 mg/ml orlistat added to solution 1 and the lipase control was the standard reaction with no inhibitors or biopolymers. All solutions were stored at 4 °C for up to 24 h.

The assay was set up over two 96 well microplates. The first contained 15 μl of solution 2 in every well. The second plate contained 180 μl of solution 1, or a concentration of biopolymer in solution 1. 12 μl of lipase solution was also added to every well (12 μl of deionised water for reagent blank well) on the second plate. The two plates were incubated for 1 h at 37 °C then 160 μl of the second plate was added to the first plate to initiate the reaction.

To calculate the percentage of lipase inhibition, the reagent blanks were subtracted from the corresponding controls or samples and the following formula was applied:

$$\text{Percentage of Lipase Inhibition} = 1 - ((\text{Polymer Sample} - \text{Inhibition Control})/(\text{Lipase Control} - \text{Inhibition Control})) \times 100$$

2.3. Lipase activity assay using olive oil as the substrate

The olive oil assay system uses a modified version of the method of Vogel and Zieve (1963). The turbidimetric method measures the reduction in turbidity that occurs following the breakdown of TAGs to free fatty acids by lipase. Olive oil, with a specific viscosity of 72.5 (±10), (specific viscosity used here is unitless as it is derived from a ratio of the oil to that of water) was used throughout the series of experiments. The olive oil was passed through aluminium oxide (80 × 15 mm deep in a glass chromatography column) to

Table 1
The alginates used in this study with some of their characteristics.

	Alginate source	Molecular weight	F(G)	F(M)	N(G > 1)	Lipase inhibition (%)
1	Mannuronan (treated with epimerase)	423,767	0.8	0.2	44	72.2
2	Mannuronan (treated with epimerase)	262,400	0.7	0.3	7	41.5
3	Mannuronan (treated with epimerase)	374,800	0.68	0.32	22	59.1
4	Seaweed (treated with epimerase)	251,220	0.68	0.32	9	56.1
5	Seaweed *L. hyperborea* SF200	387,000	0.68	0.322	16.7	55.6
6	Seaweed (treated with epimerase)	226,550	0.67	0.33	9	62.4
7	Seaweed *L. hyperborea* SF120	195,000	0.664	0.336	9.6	53.7
8	Seaweed *L. hyperborea* SF/LF	295,000	0.66	0.336	13.8	56.3
9	Seaweed *L. hyperborea* LFR5/60	34,700	0.633	0.367	9.9	52.5
10	Mannuronan (treated with epimerase)	211,833	0.62	0.38	8	60.2
11	Mannuronan (treated with epimerase)	202,000	0.62	0.38	5	58.4
12	Mannuronan (treated with epimerase)	194,833	0.54	0.46	4	60.8
13	Mannuronan (treated with epimerase)	436,767	0.52	0.48	11	62.4
14	Seaweed *Laminaria hyperborea*	260,600	0.49	0.51	9	50.2
15	Mannuronan (treated with epimerase)	456,733	0.47	0.53	0	-2.3
16	Seaweed *L. nigrescens* LF10L	75,000	0.45	0.553	4.4	10.4
17	Seaweed *L. nigrescens* H120L	397,000	0.45	0.551	5.9	28.3
18	Mannuronan (treated with epimerase)	442,367	0.44	0.56	8	64.5
19	Seaweed *L. nigrescens* LF120L	221,000	0.424	0.576	4.7	15.8
20	Seaweed *L. nigrescens* SF60	325,000	0.411	0.589	3.3	35.6
21	Seaweed *Durvillea potatorum*	241,067	0.35	0.65	4	33.5
22	Mannuronan (treated with epimerase)	438,733	0.17	0.83	0	38.5
23	Bacterial mannuronan	584,400	0	1	0	53.2

Molecular weight is in Daltons, F(G) and F(M) are the fraction of guluronate and mannuronate residues present in the polymer determined by NMR. N(G > 1) is the number of consecutive guluronate residues in the alginate polymer.