Hydrodynamic evidence for an interaction between lipase and oat beta-glucan

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Abstract

One of the ways cereal beta-glucans are considered to help suppress the production of cholesterol in humans is the interruption of lipid metabolism. One possible mechanism is the action of beta glucans in immobilising lipase - a key first-step enzyme in the breakdown of fatty acids. We explore using the powerful matrix-free technique of co-sedimentation in the analytical ultracentrifuge the properties of mixtures of porcine pancreatic lipase with a well characterised beta glucan from oat. A clear interaction between the lipase and beta-glucan is observed for a range of differing mixing ratios. The generality of this interaction is now being explored using a variety of different native and heat processed beta-glucans.

Introduction

Beta-glucans are an important water soluble fibre in human diet. Claims of the beneficial effects of oat and barley beta-glucans of the alternating beta (1-3), beta (1-4) type – see Figure 1 and Harding et al (2017) in lowering cholesterol levels have been accepted by the European food standards agency (EFSA). The mechanism(s) of action of the beta glucans have been linked to a number of causes, one of these has been an inhibition of lipolysis of fatty acid, although the precise mechanisms are still not fully understood.

One possibility – or possible contribution – to the process is a competitive interaction between beta glucans and the lipase, preventing interaction and subsequent lysis of the fatty acids. In order to explore this interaction, it is desirable to use a technique which is a matrix-free solution technique, i.e. free of columns or membranes which might otherwise pose non-inertness problems. Such a technique with its inherent separation and analysis facility is sedimentation velocity in the analytical ultracentrifuge, and this provides a useful diagnostic tool for interactions (Harding & Rowe, 2010) using the well-established assay of co-sedimentation (see Marsh & Harding, 1993; Harding & Winzor 2001). Lipases have been well studied in the past by this technique. Simpkin et al (1991) for example compared the hydrodynamic properties of lipases in the analytical ultracentrifuge from *Chromobacter viscosum* and *Pseudomonas sp*., and found very similar behaviour, with sedimentation coefficients *s*, (corrected to the density and viscosity of water at 20.0^{o}C) of (3.17$\pm$0.03)S and (2.99$\pm$0.03)S respectively and molecular weights ~36500 g/mol, consistent with results from SDS PAGE and with no evidence for significant self-associative behaviour.

In this paper we take a look at the behaviour of porcine pancreatic lipase. We take advantage also of the fact that beta glucans do not absorb in the ultraviolet whereas the lipase - absorbs at a wavelength of ~280nm. The UV absorption will only detect the lipase and the effect of any ligand which is bound to it. Although the protein lipase is well defined system, beta-glucans are polydisperse substances, with a continuous distribution of sizes.

Materials and Methods

Lipase

Porcine pancreatic lipase Type II (Sigma, Poole, Dorset UK) was dissolved and filtered and treated with PMSF (phenylmethylsulfonyl fluoride) then subjected to affinity chromatography on a column of ConA sepharose, the bound fraction was eluted with 0.4M methyl manopyranoside then dialysed. The purified protein was shown by SDS PAGE to have a molar mass of ~48000 g/mol (Figure 1) similar to that obtained for *Chromobacter viscosum* and *Pseudomonas spp.* lipases by Simpkin et al (1991) using sedimentation equilibrium in the analytical ultracentrifuge.

Beta-glucan

Oat beta-glucan was supplied by Swedish Oat Fiber AB (Väröbacka, Sweden) and is henceforth referred to as "BG1" [Guy/ Peter – could you confirm this is Oatwell90?]

Solutions

Samples for analysis were prepared in 25mM citrate buffer (pH 6.5) supplemented with 200mM NaCl.

SEC-MALS

[Guy add this if BG1 is oatwell90, or we cross refer to paper 1]

Sedimentation velocity in the analytical ultracentrifuge

The Optima XL-I was used for sedimentation velocity experiments at a rotor speed of 40000 rpm. 12mm optical path length cells at loading volumes of 400 µl were used. An 8 hole rotor was selected meaning 7 samples and one ccounterbalance could be run simultaneously under identical conditions. One of the seven cells was the lipase control at a concentration of 1.0 mg/ml, the beta glucan control at 0.4 mg/ml, and then a series of 5 mixtures. The UV absorption optical system (at 280nm) was used. Sedimentation velocity concentration versus distance records were then analysed as a function of time using the Lamm equation (as implemented in the SEDFIT algorithm of Dam & Schuck, 2004), to give distributions of sedimentation coefficient c(s) vs s, with the sedimentation coefficient in Svedbergs, S (1S = 10^{-13} sec).

Results

Characterisation of the lipase

The lipase preparation was first checked by SDS PAGE was first characterised at 20.0^{o}C by SEC-MALS [need to confirm whether BG1 is in fact Oatwell 90]. Sedimentation velocity revealed that BG1 was polydisperse with a main component at 4.4S

Sedimentation velocity

Sedimentation velocity experiments were then performed under the same conditions but at a rotor speed of 40000 rpm on lipase and BG1, and a series of 5 mixtures of lipase and BG1. UV absorption optics (at 280nm) were used. The evolution of the concentration versus distance records were analysed as a function of time using the Lamm equation (as implemented in the SEDFIT algorithm of Dam & Schuck, 2004), and the distributions of sedimentation coefficient c(s) vs s, are given in Figures 2-4.

The beta-glucan profile by itself (curve A) shows no profile since it shows no measurable uv absorption at 280nm. The lipase by itself (curve B – red profile) shows a narrow main peak at 3.5S, consistent the value seen previously by Simpkin et al (19??) for other lipases. A small amount of co-lipase is also present at a sedimentation coefficient of 1.9S and also some association product at 5S. Curves C –G show the c(s) vs s profiles for different mixtures of the lipase with the beta glucan, with the lipase concentration constant (0.5 mg/ml) but with the beta-glucan concentration ranging from 0.6 mg/ml (curve C) to 0.2mg/ml (curve G). In all cases the sharp main lipase peak (and also the co-lipase and self-association peaks) disappear, instead a broader peak commensurate with the polysaccharide beta-glucan becomes evident, indicating that all the protein has bound to the beta-glucan – or at least its 4S component, at least under these conditions and protein self-association has been suppressed.

Concluding Remarks

The protein-like sedimentation profile of lipase adopts a polysaccharide like broad profile suggesting all the lipase has bound to the beta-glucan. It would be intriguing to

now find if this corresponds to a suppression of activity. If so, it may be part of the mechanism by which beta-glucans affect the production of cholesterol.

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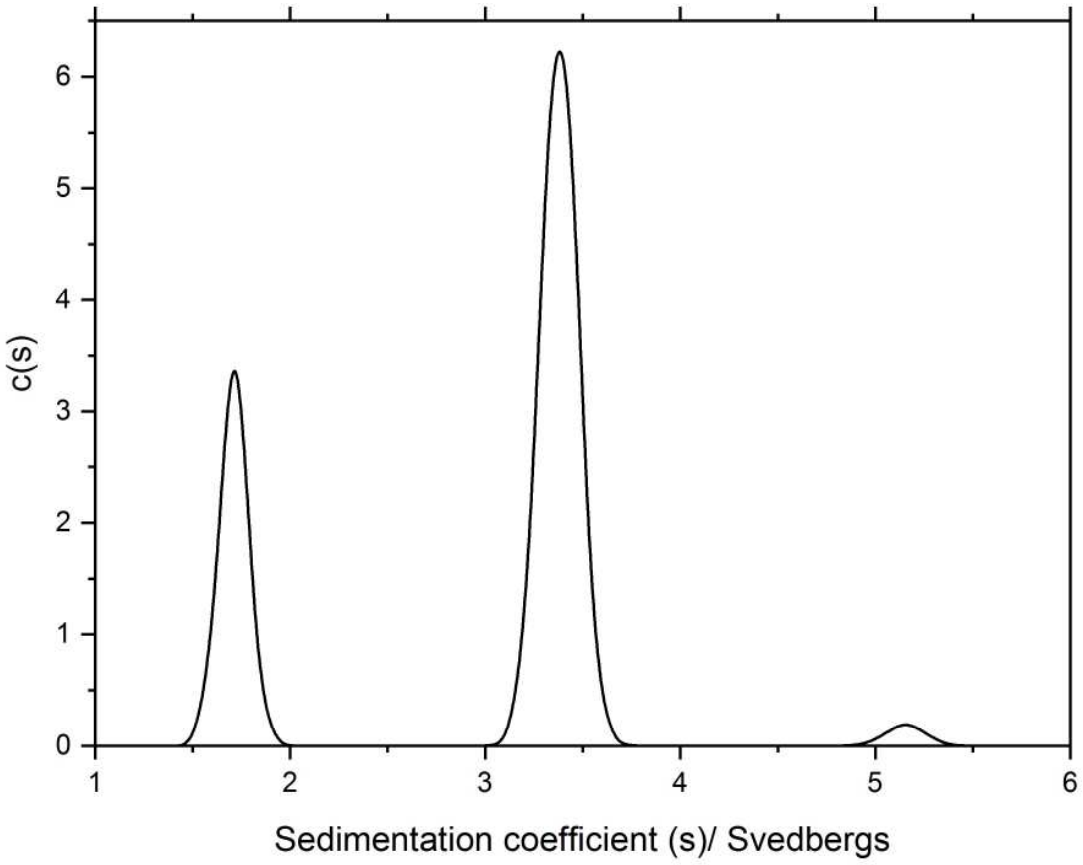
Legends to Figures

Figure 1. Sedimentation coefficient distribution c(s) vs s plots recorded at a rotor speed 40,000 rpm, 20.0^{o}C for porcine pancreatic lipase (1.0 mg/ml) recorded using (a) Rayleigh interference optics and (b) uv absorption optics at a wavelength of 280nm.

Figure 2. Sedimentation coefficient distribution c(s) vs s plots recorded at a rotor speed 40,000 rpm, 20.0^{o}C for oat beta glucan BG1 (0.4 mg/ml) recorded using Rayleigh interference optics – black trace, and uv absorption optics at 280nm (red trace);

Figure 3. Sedimentation coefficient distribution c(s) vs s plots recorded at a rotor speed 40,000 rpm, 20.0^{o}C, recorded using uv absorption optics at 280nm A: 0.4 mg/ml beta glucan only; B: lipase only, at 1.0 mg/ml. C: 0.5 mg/ml lipase + 0.6 mg/ml beta glucan, D: 0.5 mg/ml lipase + 0.5 mg/ml beta glucan, E: 0.5 mg/ml lipase + 0.4 mg/ml beta glucan, F:0.5 mg/ml lipase + 0.3 mg/ml beta glucan, G: 0.5 mg/ml lipase + 0.2 mg/ml beta glucan

Fig 1(a) top and 1(b) bottom



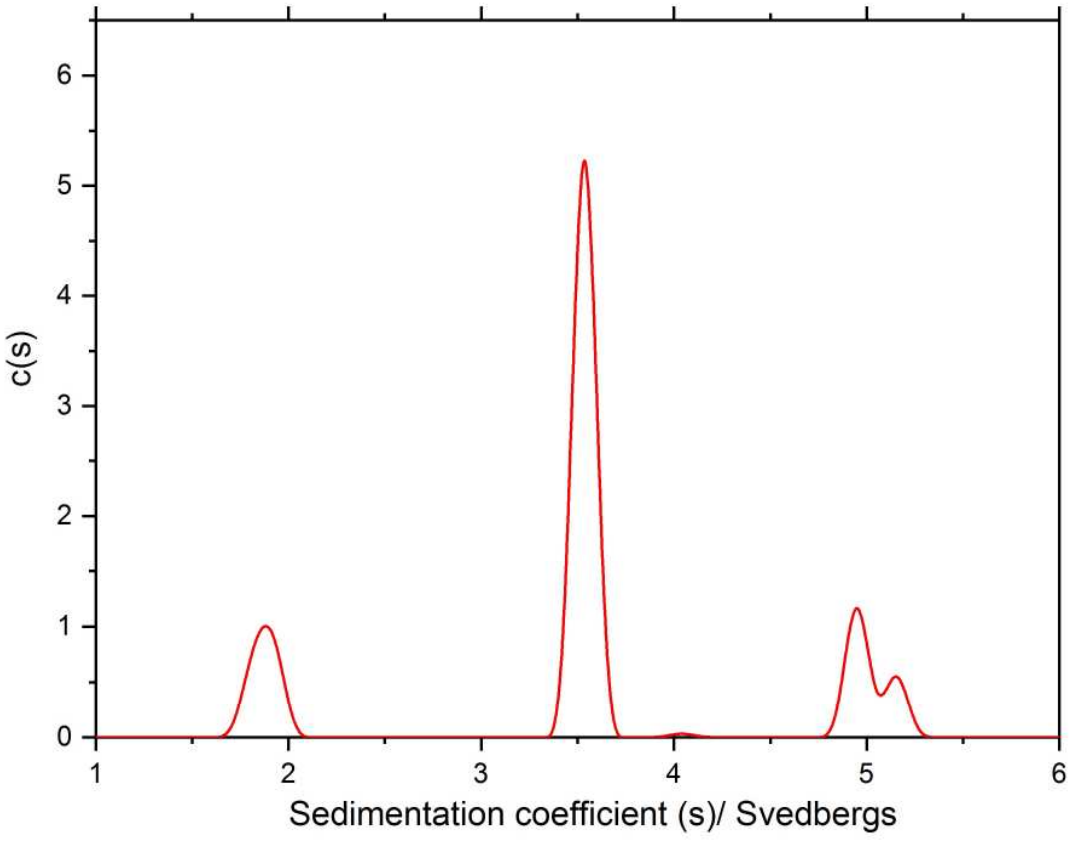


Fig 2 (top) and Fig 3(bottom)