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Endo-glucanase digestion of oat β-Glucan enhances Dectin-1 activation in human dendritic cells

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ABSTRACT

Oat β-Glucans were studied for their immunological impact before and after enzymatic digestion in order to enhance the efficacy of oat β-Glucans for application in functional foods. Oat β-Glucan is reported to have minimal impact compared to its fungal counterpart *in vitro*. Digestion with endo-glucanase enhanced its efficacy towards stimulating MCP-1, RANTES, IL-8, and IL-4 production in human dendritic cells as compared to the nondigested β-Glucan. This effect resulted from an enhanced activation of the Dectin-1 receptor. Our data suggest that the immune-stimulation was dependent on the β-(1-3) linkages and the reduced particle size of digested β-Glucans. Thus, we show that enzymatic pre-digestion of dietary fibres such as oat β-Glucan enhances its impact on specific immune receptors. We also demonstrate that particle size and/or molecular weight of oat β-Glucans and exposure of specific binding sites for the receptors might be important tools for designing efficacious functional feed and food additives.

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Abbreviations: SCFA, short chain fatty acid; PRR, pattern recognition receptor; CLEC7A, C-type lectin domain family 7 member A; DP, degree of polymerisation

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1. Introduction

Evidence is accumulating that dietary fibre intake reduces the chance on typical Western diseases (Sonnenburg & Sonnenburg, 2014) . This includes diseases with an immune component such as inflammatory bowel disease (Oliveira et al., 2013). The exact mechanisms behind this are not fully understood, but factors that were suggested to play a role are changes in gut microbiota composition and short chain fatty acid (SCFA) profiles in the intestine. These changes have been reported to attenuate immune responses as shown in several mice studies (Arpaia et al., 2013; Hansen et al., 2013; Smith et al., 2013) and clinical trials (Kiely, Ajayi, Wheeler, & Malone, 2001; Lecerf et al., 2012; Meijer, de Vos, & Priebe, 2010). More recently, it has been shown that many dietary fibres activate the so-called pattern recognition receptors (PRRs) on gut immune cells (Bermudez-Brito et al., 2015) and modulate immune responses as well as gut barrier function (Vogt et al., 2014). Insight in methods to enhance or regulate the impact of immune activating dietary fibres on PRRs can become a helpful tool in designing novel functional food and feed additives.

β-Glucan with β-(1-3) linkages from fungi is one of the first discovered dietary fibre with immune-activating properties via direct binding to PRRs (Brown et al., 2002). The β-Glucan molecule binds the PRR C-type lectin domain family 7 member A (CLEC7A) receptor also known as Dectin-1 receptor (Brown et al., 2002). β-Glucans can be found in many food and feed ingredients like yeast cell walls and oat (Knudsen, 2014). Reportedly, the immune activating capacity of oat depends on its β-(1-3, 1-4) Glucan content (Estrada et al., 1997) and oat glucans are commonly applied in feed and food products (Bartlomiej, Justyna, & Ewa, 2012). Despite these beneficial immune effects, the molecular mechanisms by which the effects of oat β-Glucan are accomplished are not completely understood. While purified oat β-Glucan has been shown to have only limited immune activating capacity *in vitro* compared to for example fungal β-Glucans (Noss, Doekes, Thorne, Heederik, & Wouters, 2013), substantial beneficial effects have been reported for oat β-Glucan *in vivo* (Murphy, Davis, Carmichael, Mayer, & Ghaffar, 2009; Volman et al., 2010). This might be explained by chemical modification of oat β-Glucans by digestion in the intestine.

The difference in β-(1-3), β-(1-6), or β-(1-4) linkages in β-Glucan molecules are considered a major reason for differences in immune activating capacity of different β-Glucans (Adams et al., 2008). β-Glucans are polymers of D-glucose linked by β-(1-3), β-(1-6), and/or β-(1-4) linkages. The lectin binding domain in Dectin-1 receptor is known to be specific for glucans with β-(1-3) and β-(1-6) linkages wherein presence of β-(1-4) along with β-(1-3) and β-(1-6) in the glucan molecule can positively influence the interaction with the Dectin-1 receptor (Brown & Gordon, 2001). The type of linkages in β-Glucan molecules as well as solubility is source dependent (Brown & Gordon, 2003). Oat β-Glucan is mainly composed of β-(1-3) and β-(1-4) linkages (Estrada et al., 1997). In addition to the linkages in the β-Glucan molecule, Dectin-1 interaction is described to be dependent on the particularity of the molecule (Goodridge et al., 2011). Particulate β-(1-3) glucans from fungal source were shown to be stronger stimulators of the Dectin-1 receptor than soluble β-Glucans. Particulate β-Glucans cluster Dectin-1 receptors on the membrane. This clustering leads to expulsion of neighbouring negative regulators such as CD45 and CD148 of Dectin-1 induced immune activation (Goodridge et al., 2011) and to a strong activation in immune cells such as dendritic cells that subsequently produce pro-inflammatory cytokines (Yokota, Takashima, Bergstresser, & Ariizumi, 2001). Soluble β-(1-3) Glucan is not able to cluster Dectin-1 receptors and fails to reduce the negative regulatory pathways resulting in lower activation patterns (Goodridge et al., 2011).

In the present study, we hypothesized that enzymatic degradation of oat β-Glucans into oligomers leads to changes in particle size and will impact the oat β-Glucan induced immune responses in human dendritic cells by changing the binding kinetics to the Dectin-1 receptor. The interaction of the polymer and enzymatic digested molecules were studied with two splice variants of human Dectin-1, i.e. Dectin-1A and Dectin-1B. Dectin-1A has a stalk region between the extracellular domain and the transmembrane domain which is absent in Dectin-1B with possible effects for immune activation (Willment, Gordon, & Brown, 2001; Yokota et al., 2001). Additionally, the relationship between activation patterns of oat β-Glucan with particle size was studied.

2. Material and methods

2.1. *β-Glucans and enzymatic modification*

Commercial oat β-(1-3, 1-4) Glucan (medium viscosity) was purchased from Megazyme (Wicklow, Ireland). Endo-glucanase from *Aspergillus niger* was provided by DSM Food Specialities (Delft, the Netherlands). β-Glucan (10 mg/mL) was suspended in 10 mM sodium acetate buffer (pH 5.0). Enzyme was added at 0.5 μL/mg β-Glucan. The mixture was incubated at 37 °C for 12 hours in a head-over-trail rotator. Enzyme was inactivated by boiling the mixture for 10 min. Solutions (1.0 mL) were filtered through a 0.22 μm filter membrane for chemical characterization. Other solutions were lyophilized for further analysis.

β-Glucan samples were tested using a LPS specific ELISA (ELISA kit from Clone-cloud corp., Houston, TX, USA). The LPS concentration was lower than the detection levels of 4 ng/mL. None of the β-Glucan samples applied in this study were responsive at this concentration.

2.2. *Molecular weight distribution measurement by HPSEC*

To monitor the molecular weight change of β-Glucan after enzyme treatment, high performance size exclusion liquid chromatography (HPSEC) was performed on an Ultimate 3000 HPLC system (Thermo Scientific, Waltham, MA, USA) using a PWX-guard column (6 mm i.d. × 40 mm, Tosoh Bioscience, Tokyo, Japan) and three TSK-gel columns connected in series (4000, 3000, and 2500 SuperSW, 6 mm i.d. × 150 mm per column, Tosoh Bioscience, Tokyo, Japan). A sample of 10 μL (2.5 mg/mL in 10 mM sodium acetate buffer, pH 5.0) was injected and eluted with 0.2 M sodium nitrate at a flow rate of 0.6 mL/min at 55 °C. The HPLC system was controlled by Chromeleon version 7. Detection was achieved with a refractive index (RI) detector Shodex R101 (Showa Denko, Japan). The molecular mass distribution