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Effect of apotransferrin, lactoferrin and ovotransferrin on the hydroxyl radical mediated degradation of beta-glucan

Audrey M. Faure, Laura Nyström *

ETH Zurich, Institute of Food, Nutrition and Health, Schmelzbergstrasse 9, 8092 Zurich, Switzerland

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ABSTRACT

Beta-glucan is a polysaccharide widely accepted and used as a functional ingredient due to its positive effects on human health. However, beta-glucan is readily degraded in aqueous systems in presence of a hydroxyl radical generating system such as ascorbic/iron(II). In the present study, we tested whether iron binding proteins; apotransferrin, lactoferrin and ovotransferrin; could prevent the hydroxyl radical mediated degradation of beta-glucan. The radical formation was investigated by ESR spectroscopy and the polysaccharide degradation was monitored by the viscosity loss of the solutions. Apo-transferrin increased the formation of hydroxyl radicals and this related with a faster degradation of beta-glucan. Lactoferrin did not have any effect on the ascorbate induced degradation of beta-glucan, whereas ovotransferrin completely inhibited the hydroxyl radical generation by a system containing ascorbic acid and iron (II). However, the presence of ovotransferrin in beta-glucan decreased the viscosity of the solution, which was accompanied by the apparition of a precipitate, indicating a potential interaction between the protein and beta-glucan. FT-IR analyses indicate the presence of beta-glucan and ovotransferrin in both precipitate and supernatant, as well as the occurrence of interactions between the two compounds. This study reveals that ovotransferrin is a promising candidate for inhibiting the formation of ascorbate/iron(II) induced hydroxyl radicals in beta-glucan solutions.

1. Introduction

Cereal (1 → 3), (1 → 4)-β-D-glucan is a dietary fiber found pre-dominantly in the endospermic and aleuronic cell walls of barley and oat (Lazaridou & Biliaderis, 2007). It is a homopolysaccharide composed of blocks of consecutive β-(1 → 4) linked D-glucopyranosyl segments separated by single β-(1 → 3)-linkages (Lazaridou & Biliaderis, 2007). In the last decades, beta-glucan has gained increased interest from the food industry as a functional food ingredient *due* to its various health *beneficial effects*. Studies have demonstrated that beta-glucan has high potential for the prevention and treatment of chronic diseases, especially diabetes and associated cardiovascular disease (Wood, 2007; Wood, 2010). This results from beta-glucan's ability to regulate blood glucose levels, and decrease cholesterol in case of hypercholesterolemia (Wood, 2007). However, beta-glucan fortification of foods, especially liquid food products, remains a challenge for the food industry due its susceptibility to degradation in presence of iron (Faure, Andersen, & Nyström, 2012; Faure, Werder, & Nyström, 2013; Kivelä, Gates, & Sontag-Strohm, 2009; Mäkelä, Sontag-Strohm, & Maina, 2015). Indeed, studies have shown that the presence of iron in beta-glucan solution induces a hydroxyl radical mediated degradation of the polysaccharide. Therefore, a method to capture iron ions and make them unable to generate hydroxyl radicals could be a way to prevent beta-glucan degradation in aqueous food products.

Previous studies have demonstrated that the mere presence of iron(II) in pure beta-glucan solutions causes the viscosity loss of the solutions, which indicated that the polysaccharide suffered from degradation (Faure, Knüsel, & Nyström, 2013; Faure et al., 2013; Kivelä, Henniges, Sontag-Strohm, & Potthast, 2012). Additionally, ESR studies have shown that iron(II) in beta-glucan solutions promoted the formation of hydroxyl radicals, which were responsible for the degradation of the polysaccharide. Iron(II) can induce the formation of hydroxyl radicals through a three step chain reaction beginning with its autoxidation. The superoxide radical resulting from Reaction (1) can self-dismute into hydrogen peroxide (Reaction (2)), the latter then react with the remaining iron(II) through the Fenton Reaction (3), producing hydroxyl radicals.

$$Fe^{2+} + O_2 \rightarrow Fe^{3+} + O_2^{\cdot-} \quad (1)$$

$$2O_2^{\cdot-} + 2H^+ \rightarrow H_2O_2 + O_2 \quad (2)$$

* Corresponding author.
E-mail address: laura.nystroem@hest.ethz.ch (L. Nyström).

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$$Fe^{2+} + H_2O_2 \rightarrow {\cdot}OH + {}^{-}OH + Fe^{3+} \quad (3)$$

The introduction of a reducing agent, such as ascorbic acid, into an iron(II) containing beta-glucan solution enhances the formation of hydroxyl radicals and thereby accelerates beta-glucan degradation. This is due to the fact that ascorbic acid can convert iron(III) back to iron(II) (4), and therefore provides a continuous supply of iron(II) for the Fenton reaction.

$$AH_2 + Fe^{3+} \rightarrow A + 2H^+ + Fe^{2+} \quad (4)$$

In a previous study, we have confirmed the involvement of superoxide radical and H_2O_2 in both iron(II) and AH_2/iron(II) induced degradation of beta-glucan, and therefore demonstrated that iron ions plays a key role in the formation of hydroxyl radical (Faure et al., 2013). Thus using compounds that can capture iron ions (iron(II) or iron(III)) selectively could be a good strategy to prevent beta-glucan degradation in solution.

Iron chelators such as citric acid and phytic acid have been tested in beta-glucan solution containing AH_2/Fe^{2+} to prevent beta-glucan degradation, however none of these compounds demonstrated a protective effect against hydroxyl radical mediated oxidative cleavage (Faure, Münger, & Nyström, 2012; Kivelä, Nyström, Salovaara, & Sontag-Strohm, 2009). Some studies have shown that iron-binding proteins (transferrins) might have a protective effect against the generation of hydroxyl radicals by binding the iron from the aqueous medium. Transferrins are glycoproteins, which bind iron ions selectively and very tightly, and which are naturally found in food products. In the present study, we evaluated apo-transferrin, lactoferrin and ovotransferrin as iron binding compounds to prevent iron-induced degradation of beta-glucan. Therefore to assess the effect of transferrins on ·OH-driven beta-glucan degradation, ESR spin trapping method was used to evaluate the efficiency of transferrins in inhibiting the formation of ·OH in AH_2/Fe^{2+}-treated barley beta-glucan solution. Moreover, they were further evaluated regarding their effectiveness to inhibit the resulting losses in viscosity over time.

2. Materials and methods

2.1. Materials

High viscosity barley β-glucan (purity > 97%) was purchased from Megazyme (Ireland). The proteins bovine apotransferrin (purity ⩾ 98%), lactoferrin from bovine milk (purity ⩾ 85%) and conalbumin (ovotransferrin) from chicken egg white (substantially iron free) were purchased from Sigma–Aldrich (USA). The first two were stored between 8 °C, and the latter was stored at −20 °C. Ascorbic acid (⩾99.5%) and sodium bicarbonate (⩾99.0%) were bought from Fluka (Germany). Iron(II) sulfate heptahydrate (>99.0%) was purchased from Sigma–Aldrich Chemie GmbH (Germany). The spin traps POBN (α-(pyridryl *N*-oxide)-*N*-tert-butylnitrone; 99%); DMSO (dimethyl sufoxide, CHROMASOLV® Plus, for HPLC, ⩾99.7%) and the reference TEMPO (2,2,6,6-tetramethylpiperidine 1-oxyl, free radical, sublimed, 99%) were from Sigma–Aldrich (USA) and were stored at −20 °C.

2.2. Preparation of the β-glucan solution

The 0.6% β-glucan solution was prepared from dry high viscosity barley β-glucan, by adding 0.6 g beta-glucan in a 100 mL volumetric flask, which was filled with most of the Milli-Q-water. The solution was heated in a water bath 3 h at 80 °C under continuous shaking, and after complete dissolution, the flask was filled up to 100 ml. The pH of the solution was adjusted to 6.0 by adding hydrochloric acid (0.1 M) and sodium hydroxide (0.1 M).

2.3. ESR based assay to assess antioxidant capacity of the transferrins

The formation of free radicals in the different models was monitored using ESR (electron spin resonance) spin trapping method. The spin trapping method used consisted in an indirect detection of the hydroxyl radicals using POBN in combination with DMSO. We have previously showed that this method was suitable for the detection of hydroxyl radical in beta-glucan solution (Faure et al., 2012). The mechanism involved is an oxidation of DMSO by ·OH which leads to the formation of methyl radicals (·CH_3), which are further trapped by POBN, resulting in the formation of stable POBN–CH_3 spin adducts detectable by ESR. Stock solution of POBN (4 M) was prepared by dissolving a certain amount of spin trap in Milli-Q water. Using this method the rate of ·OH formation in beta-glucan solution was investigated over time. The protein powders were added first to the beta-glucan containing iron(II) (50 μM), followed by the addition of POBN and DMSO. Ascorbic acid (10 mM) solution was always mixed last to the system because it initiates ·OH formation. POBN together with DMSO were used at final concentrations of 80 mM and 2% (v/v), respectively.

2.4. Electron spin resonance (ESR) measurements

The samples were loaded in 50 μL micropipettes (Brand GmbH, Wertheim, Germany) and the ESR spectra were recorded with a High Sensitive Benchtop EPR Spectrometer MiniScope MS300 (Magnettech, Berlin, Germany) at room temperature. The settings used were as follows: B0-field, 3350 G; sweep width, 100 G; sweep time, 30 s; steps, 4096; number of passes; 4, modulation frequency, 1000 mG; microwave attenuation, 10 dB; receiver gain, 900. The relative ESR signal corresponding to the content of spin adducts was obtained by calculating the ratio between the peak-to-peak-amplitude of the first doublet in the ESR signal of POBN adduct and the peak-to-peak-amplitude of the first singlet in the ESR signal of a TEMPO solution (2 μM in H_2O). TEMPO was used as a standard and was measured in triplicate on each day of measurements.

2.5. Rheology measurements

The viscosity measurements were performed in an AR-2000 rheometer (TA Instruments, USA) with computer control, using cone geometry with 20 mm radius and an angle of 2°. The parameters used in these analyses were: temperature, +20 °C; gap, 59 μm and shear rate range, 20–2000 s^{-1}. The viscosity of each sample was assessed every 0, 2, 5, 24 and 168 h, similarly to the ESR measurements.

2.6. FTIR analysis to investigate the interaction between ovotransferrin and barley β-glucan

The interaction between ovotransferrin and barley β-glucan was studied by mixing 7.5 mL of barley β-glucan solution (0.6%) and 45 mg of ovotransferrin. After two weeks, the solution was centrifuged (Centrifuge 5810R, Vaudaux-Eppendorf AG, Switzerland) according to the parameters: temperature, 24 °C; speed, 4000 rpm; time, 15 min. Then, the supernatant and the precipitate were separated and freeze-dried (lyophilizer Lyolab BII LSL Secfroid, Switzerland). Fourier-Transform Infrared (FTIR) spectra of the solid samples (freeze-dried) were recorded at room temperature with a Varian 640 FTIR spectrometer and a MKII Golden Gate single Attenuated Total Reflection (ATR) system.