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ScienceDirect
Carbohydrate Research 342 (2007) 1434–1441
Carbohydrate RESEARCH

Carbanilation of cereal β-glucans for molecular weight determination and conformational studies

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Received 22 December 2006; received in revised form 3 April 2007; accepted 6 April 2007
Available online 21 April 2007

Abstract—Cereal β-glucans can form aggregates in aqueous solution. The presence of aggregates in cereal β-glucan solutions led to inaccurate determination of molecular weights and it was believed that intermolecular hydrogen bonding caused the aggregation. To eliminate aggregates, a carbanilation method for molecular weight determination of cereal β-glucans was developed. Wheat β-glucan samples were selected for investigation. The carbanilation method can prevent intermolecular hydrogen bonding by blocking hydroxyl groups with phenyl carbamate groups. The carbanilates of cereal β-glucans were prepared by the reaction of cereal β-glucans with phenylisocyanate catalyzed by DMSO and pyridine. To avoid degradation during the carbanilation reaction, relatively mild conditions were used, which led to incomplete substitution (DS: ~2). However, after the carbanilation reaction, the carbanilates dissolved completely in 1,4-dioxane solution without any detectable aggregates, which allowed accurate molecular weight determination. The degree of substitution (DS) of carbanilates was determined by both a nitrogen content method and an FT-IR method. The FT-IR method proved to be the more effective for DS estimation. Using this method, the converted molecular weights of cereal β-glucans were in good agreement with the results measured in 0.5 M NaOH solution, which previously was shown to be a good solvent for cereal β-glucans. After the carbanilation reaction, conformational changes of carbanilates were studied by static and dynamic light scattering techniques. The fractal dimension ($d_f = 2.27$) and the structure sensitive parameters ($\rho > 2$) suggested a porous globular structure for partially carbanilated β-glucans.

Keywords: Cereal β-glucans; Carbanilate; Molecular weight; Aggregates; Degree of substitution; Conformation; FT-IR; Light scattering

1. Introduction

(1→3)(1→4)-β-D-Glucans are cell wall polysaccharides located in the cereal endosperm and aleurone cells. Cereal β-glucans are linear homoglucans of D-glucopyranose arranged as blocks of consecutive (1→4)-linked β-D-glucose residues separated by single (1→3)-linkages.[1] The ratio of oligosaccharide segments with two consecutive (1→4)-linkages (trisaccharides) to those with three consecutive (1→4)-linkages (tetrasaccharide) is a characteristic structural indicator of cereal β-D-glucans, which follows the order of wheat (4.2–4.5), barley (2.8–3.3), and oat (2.0–2.4).[2] This trend corresponds with the differences of their conformational and physical properties of cereal β-glucans, such as, chain stiffness, solubility, viscosity, and gelling properties. To study the structure–function relationships of cereal β-glucans, it is essential to obtain an accurate measurement of their molecular weights. Light scattering is one of the important experimental tools for the determination of the absolute molecular weights of polymers due to its high sensitivity for detecting large molecules at low concentrations. However, it is a challenge to accurately determine the molecular weights of cereal β-glucans by light scattering techniques due to severe aggregation in aqueous solutions. It is believed that the aggregation

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0008-6215/$ - see front matter © 2007 Published by Elsevier Ltd.
doi:10.1016/j.carres.2007.04.005

of cereal β-glucans is mainly caused by intermolecular hydrogen bonding and the interference of macromolecular aggregates in molecular weight determination has been reported by many groups.[3–6] A number of physical and chemical methods have been used to eliminate the aggregates such as filtration,[7–9] heating, centrifugation,[3] or the use of different solvents.[10] However, only limited success was achieved using these methods due to the lack of effective and direct way to evaluate their effectiveness. For example, the amount of β-glucan aggregates were too small to be detected by osmotic pressure measurements used by Vårum et al.[3] The dynamic light scattering (DLS) method allows determination of size distributions over a wide range of size where the size of macromolecular aggregates are comparable to that of un-aggregated counterparts. By applying the dynamic light scattering technique to monitor the presence of aggregates, a method for eliminating the aggregates using NaOH solutions has been developed by our group, which allows the accurate determination of molecular weights.[6]

In the present study, a carbanilation method was used to chemically substitute the hydroxyl groups of cereal β-glucans by phenyl carbamate groups to prevent the formation of hydrogen bonding and hence, aggregation. To the best of our knowledge, this idea was applied for the first time to amylose and cellulose in 1961 by one of the authors[11] who compared the solution properties of these two isomeric tricarbanilated polysaccharides, which were determined by static light scattering and viscometry in dioxane, pyridine and acetone. The conformational properties were later confirmed and completed by dynamic light scattering.[12] In the meantime, the reaction of phenylisocyanate has become a routine technique for natural and bacterial celluloses.[13–21] This method may provide an alternative method for eliminating aggregation of cereal β-glucans and it is expected that the bulky derivatives will exert a similar influence on the solution properties of various cereal β-glucans. The carbanilates of cereal β-glucans could be used to investigate the conformational differences between oat, barley, and wheat β-glucans, which could provide useful information for understanding structure–conformation relationships. However, due to steric hindrance and intramolecular hydrogen bonding between neighboring carbanilate groups along the backbone chain[17] a change in the conformation of the cereal β-glucan chains may be expected, for example, considerable deviations from the chain stiffness of cellulose tricarbanilates.[22] In the present study, the carbanilation method proved to be somewhat more involved than for cellulose and required optimization. The molecular weights were determined, and the changes in the conformational properties after carbanilation were investigated. An FT-IR method was developed to determine the degree of substitution.

2. Results and discussion

2.1. Carbanilation of cereal β-glucans

The reaction conditions of cellulose with phenyl isocyanate were studied extensively, and it was reported that the fully trisubstituted products could be easily prepared.[19–21,23] However, it is also reported that degradation occurs upon derivatization of cellulose depending on reaction time, temperature, and co-reactant. Furthermore, an unskilled precipitation procedure sometimes resulted in a loss of low molecular weight fractions up to 20%.[21] These phenomena would cause inaccurate molecular weight determination of native unfractionated cereal β-glucan derivatives.

In some cases, it is difficult to achieve full substitution of cereal β-glucans without degradation. The steric hindrance may prevent the full substitution as well. For the purpose of determining molecular weight, it is not necessary to achieve complete substitution as long as the final products can dissolve fully dispersed in 1,4-dioxane (no aggregates), which allows accurate molecular weight determination by light scattering. To prevent degradation problems, relatively mild reaction conditions were used in the present study, resulting in partially substituted carbanilate products.

To correctly convert the results from these derivatives to the molecular weights of un-substituted cereal β-glucans, it is essential to determine the degree of substitution of cereal β-glucan carbanilates accurately. It is worth noting that the solvents used for the carbanilation reaction play an important role in the reaction rate and degradation. From the results of cellulose carbanilation, the efficacy of the solvents for the carbanilation reaction decreases in the order of dimethylsulfoxide (DMSO) > pyridine > *N*,*N*-dimethylformamide (DMF) ≈ *N*,*N*-dimethylacetamide (DMA).[19] The explanations for this phenomenon are controversial. It was proposed that solvents with high dielectric constants[24] or electron donor numbers[25] were particularly effective for the reactions. However, Evans et al. concluded that the rate of dissolution of cellulose during the carbanilation reaction is probably governed by the rate and extent of swelling of the cellulose in solvents.[20] Our result for cereal β-glucan carbanilation supports the latter explanation.

Cereal β-glucans swell well in DMSO at 60 °C, but not in pyridine, even at higher temperatures. On the other hand, pyridine is an important additive to catalyze the reaction. However, the use of DMSO caused severe degradation during the carbanilation reaction of cereal β-glucan samples (data not shown). Therefore, pyridine was used as the carbanilation solvent in the present study. To promote the swelling of cereal β-glucans in pyridine, a small amount of DMSO, at a concentration below which degradation occurred, was added. However, the addition of DMSO also promoted the