Journal of Chromatography A, 1205 (2008) 60–70

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Toward a full characterization of native starch: Separation and detection by size-exclusion chromatography

Ngoc-Ly Hoang, Antonin Landolfi, Anastasiya Kravchuk, Etienne Girard, Jonathan Peate, Javier M. Hernandez[1], Marianne Gaborieau, Olena Kravchuk, Robert G. Gilbert, Yohann Guillaneuf[2], Patrice Castignolles*

University of Queensland, CNAFS/SLCAFS, Hartley Teakle Building 83, Brisbane , QLD 4072, Australia

ARTICLE INFO

Article history:
Received 5 April 2008
Received in revised form 24 July 2008
Accepted 25 July 2008
Available online 7 August 2008

Keywords:
Size-exclusion chromatography (SEC)
Separation
Repeatability
Labeling
UV detection
Starch

ABSTRACT

The structure of starch molecules is relevant to nutrition and industrial applications. Size-exclusion chromatography (SEC, also known as GPC) of native starch generally suffers non-satisfactory repeatability and reproducibility of the dissolution and separation. This work combines two polar organic solvents: dimethylsulfoxide for complete dissolution and dimethylacetamide to limit shear degradation. The separation is as repeatable as that of polystyrene standards performing dissolution and separation at 80 °C. Successful covalent-labeling on the glucose unit is claimed to be published here for the first time in non-degradative conditions and allows the use of UV detector with significantly higher sensitivity than with a refractometer.

1. Introduction

Diet and nutrition have become very serious issues in the last 50 years, as the world faces the spreading of diet-related chronic disorders such as diabetes, obesity and cardiovascular diseases. Starch consumption represents over 50% of the average caloric intake in developed countries [1] and up to 90% in developing ones, thus giving it a key role in diet issues and nutrition-related diseases. It is also important for animal nutrition, in particular for farmed animals and thus another component of human diet. There are many measures of the 'digestibility' of starch, such as glycemic index [2]; while no single simple measure is sufficient to serve unambiguously as a criterion for 'good' nutrition, comparing starch samples with different digestibility indices is a significant step to establish structure–property relationships. Equally as important as measuring digestibility of a food (by whatever means) is a description of the structure of the component starches. An important step in the full characterization of starch is to be able to perform meaningful comparative studies.

Even though starch is the simplest type of natural polymer in that there is only one monomeric unit, glucose, it has an extremely complex structure, with structural features over a vast range of scales [3,4], from a few nanometers for individual branches through to the millimeter size of a grain, built up from an intricate branched structure. Up to six structural levels [3] can be identified. The present paper concerns the second of these levels: the branched structure of an individual native starch molecule.

Starch has two main components: the amylose (slightly long-chain branched glucan) [5] and the amylopectin (highly short-branched glucan). Each component has a broad distribution of both size and molar mass [5–7]. While it is a straightforward matter to separate unbranched homopolymers and characterize their structure by its molar mass distribution, this is no longer the case for a complex branched polymer such as starch. A range of different molar masses can have the same size, and any structural characterization must include some quantification of the branching, including connectivity. The dimensionality of starch samples is thus expected to be at least three for amylose and more for amylopectin. Measurement of this structural complexity in a way that can be useful for structure–property relations is a major problem.

* Corresponding author. Tel.: +61 7 3365 2025; fax: +61 7 3365 1188.
E-mail address: p.castignolles@uq.edu.au (P. Castignolles).
[1] Present address: Department of Neurobiology, Max-Planck-Institute for Biophysical Chemistry, Am Fassberg 11, D-37070 Goettingen, Germany.
[2] Present address: Université Aix-Marseille I, Laboratoire Chimie Provence, UMR 6264, case 542, Avenue Escadrille Normandie Niémen, site de St Jérôme, 13397 Marseille Cedex 20, France, Tel.: +33 4 91 28 87 33.

0021-9673/$ – see front matter.
doi:10.1016/j.chroma.2008.07.090

Size-exclusion chromatography (SEC) is the most developed, understood and commonly used method to separate synthetic polymers [8–10]. SEC separates on size, which for a complex branched polymer does not have a unique relation to molar mass [9,11,12], specifically, the hydrodynamic volume [13–15]. Multiple-detection SEC [11,16,17] provides tools, which can be used to provide structural information on starch. However, SEC separation must be free of artifacts, and in particular must fully separate all the individual starch molecules in the sample, without degradation. For starch in particular, this is a very hard task.

Initial eluents for SEC of native starch were aqueous based [5,18,19] and they are still the most commonly used, including acidic eluents [5,20], basic eluents [6,7,18] and neutral eluent [19,21–24]. Full characterization by SEC requires complete dissolution of the analyte with minimal degradation. Samples tend to crystallize (retrogradation) and thus dissolution is not trivial [21,25] and retrogradation certainly can occur readily in aqueous eluents (e.g. [26]).

Different strategies have been used to force the dissolution in aqueous eluent: heating at high temperatures (above 100 °C) and/or high pressure (autoclave treatment), microwave heating and sonication. Degradation is regularly observed or suspected [6,21]. Retrogradation is spontaneous and non-repeatable [6,18,27] and is thus likely to lead to poor repeatability of elution volumes and detector signals; this repeatability has never been quantified although it has been described as "good" in alkaline solvents [6] (although this reference did not show chromatograms) and in acid solvents [28] (although the chromatograms in Fig. 3 of this reference do not seem to show a reproducibility that would be regarded as optimal).

Polar organic solvents have been applied to overcome the aforementioned problems. Striegel et al. showed that dimethylacetamide/lithium chloride (DMAc/LiCl) is a good solvent and eluent to separate polysaccharides [29,30]. LiCl forms a macro-cation with DMAc, which can further interact with the polysaccharide to dissolve it. Their work also extended into the use of DMAc/LiCl as an eluent for SEC [30], as did other work by Politz et al. [31]. Unfortunately, the preparation of starch samples still requires heating at high temperatures (including 1 h at 150 °C) to ensure dissolution of the polysaccharides. This may cause degradation of the sample. Another polar organic solvent that has been used for SEC of starch is dimethylsulfoxide (DMSO) [27], used not infrequently in the literature (e.g. [25,32]). DMSO can completely solubilize starch granules [18], and when dry, complete dissolution is expected after 20–30 min at 80 °C according to an NMR study of the kinetics of dissolution of some rice starches by some of the authors [33] although this needs to be adapted for each sample and 29 h have been allowed to dissolve potato starches [25]. Starch is usually dissolved in a mixture of DMSO and water (e.g. [22,24,34,35]). Note that the presence of water in DMSO slows down the kinetics of dissolution of starch [33], even though it accelerates the loss of crystallinity [36,37] of the starch granules. Unfortunately, the high viscosity of DMSO makes its use as an eluent tedious, as it will build up pressure inside the column. Even more importantly, the high viscosity generates shear scission forces that are likely to degrade high molar mass materials, such as amylopectin. Degradation from shear scission has been recorded for higher molar masses of polystyrene in tetrahydrofuran [38,39], in toluene, with $\overline{M}_w$ above 5×10^6 (flow rate = 0.5 mL min^{-1}, concentration = 0.25 g L^{-1}) and for polyethylene [40]. Varying experimental conditions can modify the extent of degradation, and while it can be minimized, it may be impossible to avoid it entirely [41,42].

Full characterization of amylopectin, and possibly amylose, should thus be performed in an eluent containing DMSO to ensure minimal degradation and quantitative dissolution and another co-solvent decreasing the viscosity of the eluent. Although water is less viscous than DMSO, introducing low levels of water in DMSO leads to an increase of viscosity because the mixture is not ideal [43].

The present paper gives various evolutionary advances on these techniques, with an aim to improve starch structural characterization. Building on advances by Striegel, we employ an eluent combining DMSO and DMAc towards overcoming some of the problems (poor repeatability or reproducibility, biased injection, low recovery, shear degradation, etc.) that have plagued this endeavor in the past. LiBr is added to prevent adsorption of starch onto the SEC columns [44]. To determine whether our system allows us to perform meaningful comparative studies [45], we study the separation and detection repeatability (experiments with the same method on identical test material in the same laboratory by the same operator using the same equipment within short intervals of time [46]) and reproducibility (experiments with the same method on identical test material in different laboratories with different operators using different equipment [46]).

The similarity of the refractive indices of organic polar solvents and starch [17] results in a low RID sensitivity, which in turn makes quantification of the signal difficult because of a low signal-to-noise ratio. This difficulty is further increased by the fact that amylopectin has a very high molar mass, and accordingly must be injected at low concentration. Thus the detection must be improved in the least degradative conditions possible. Labeling starch with UV-active or fluorescent groups is a good method to increase the sensitivity of the detection. Due to the selectivity (specific wavelength for the adsorption) and high extinction coefficient available with appropriate chromophores, UV or fluorescent spectroscopy is specific and highly sensitive towards the active groups. To help overcome the problem of detector sensitivity, we propose what appears to be the first means of randomly labeling starch on the backbone of the polysaccharide, i.e. by reaction on any anhydroglucose unit, to have access to the mass distribution with high sensitivity.

2. Experimental

2.1. Materials

N,*N*-Dimethylacetamide (DMAc, 99.8%, HPLC grade) and dimethylsulfoxide (DMSO, HPLC grade) were purchased from Sigma–Aldrich, acetonitrile and isopropanol (both AR grade) from Lab-scan Analytical Science, DMSO–d_6 (99.9% D) and acetonitrile–d_3 (99.8% D) from Cambridge Isotope Laboratories Inc. When used for starch dissolution, DMSO and DMSO–d_6 were dried using 3 Å molecular sieves (Sigma–Aldrich) before use. Lithium bromide (LiBr, Sigma–Aldrich) was dried in a vacuum oven at 160 °C under vacuum for 10 h and then stored in a dessicator. A 0.2-μm membrane filter (hydrophilic Teflon, Millipore) was used to remove any particulates prior to use. The starch used was rice starch (Sigma–Aldrich, S-7260, 11.6 wt.% moisture content); its amylose content was found to be 36% by the iodine binding method (see supporting information). Triethylamine (Et_3N) and *N*,*N*-disuccinimidyl carbonate (DSC) were purchased from Merck and Fluka, respectively. Toluene (HPLC Grade), 2-naphthylamine (98%) and 2-(2-naphthyl)-ethylamine (97%) were supplied by Sigma–Aldrich. The poly(vinyl alcohol) was purchased from BDH Chemicals (specified by the supplier to have a molar mass of 14,000 g mol^{-1}).

Note that when DMSO is used, any contact with rubber should be carefully avoided (see [33] and supporting information). Moreover, DMSO is sometimes considered as toxic [20] although its safety data sheet (MSDS) does not state this. DMAc is toxic and requires special care.