



Short communication

Rapid method to determine the molecular weight of dextrans and dextrans

Kamran Alvani^{*}, Xin Qi, Richard Tester

Glycologic Limited, c/o Caledonian University, Cowcaddens Road, Glasgow G4 0BA, UK

ARTICLE INFO

Article history:

Received 8 May 2009

Received in revised form 15 June 2009

Accepted 22 June 2009

Available online 1 July 2009

Keywords:

Dextran

Dextrin

 β -Limit dextrin

Molecular weight

ABSTRACT

A rapid method was developed to determine the molecular weight (M_n) of β -limit dextrin and dextrans (*Leuconostoc mesenteroides*) using a reducing power approach. The M_n of the β -limit dextrin was also estimated from high performance liquid chromatography (HPLC). Chromatograms were pre-calibrated with the dextrans. The three dextrans had a M_n of 2.09, 2.40 and 2.63×10^5 using the reducing method and 4.80, 5.90 and 2.80×10^5 by HPLC. The method could be employed to estimate M_n of dextrans where chromatographic systems were not available.

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1. Introduction

Dextrans are water-soluble, commonly available food ingredients that are made by enzyme-catalysed hydrolysis of starch. More common on the market are maltodextrins (Sikora, Schilling, Tomasik, & Li, 2002). Dextrans are depolymerised and sometimes restructured starch molecules where the modifications are usually induced in native starch granules. These molecules are commonly produced by the dry heating of unmodified starches although the term also includes products resulting from enzyme or acid-catalysed hydrolysis of wet starches (Budavari, O'Neil, Smith, Heckelman, & Kinneary, 1996). Dextrans are assumed usually to be polysaccharides containing some branching (or be part of a mixture containing branched molecules) due to the presence of some α -(1,6) bonds amongst the bulk of the glucan bonds which are α -(1,4) bonds. Usually dextrans comprise a polydisperse mix rather than a distinct single molecular species. Enzymes may also be used to make dextrans, especially α -amylase (to make α -dextrans). More extensive hydrolysis leads to the production of glucose syrup and glucose itself (where amyloglucosidase may also be used to enhance the conversion to glucose). The β -limit dextrans (Tester, 2005) are large molecular weight materials derived from β -amylase hydrolysis of amylopectin; having approximately 40% of the native amylopectin molecular weight. These dextrans are easily dispersed in water and have been developed for pharmaceutical applications (Tester, 2005). Theoretically the reducing power can be used to estimate the molecular weight on a number basis (M_n) since each molecule has a single reducing group (not the

cyclodextrins). The following work represents a method developed to determine the molecular weight (actually M_n) of β -limit dextrin although it could be applied to other dextrans and dextrans. Dextrans were used as standards. They are biocompatible biopolymers comprised of glucose units linked predominantly α -D-(1,6).

2. Materials and methods

2.1. Materials

All chemicals used were reagent-grade or better. Dextran standards were purchased from Fluka, Gillingham, UK (Table 1) and β -limit dextrans were provided by a commercial supplier.

2.2. Chromatographic method

The High Performance Size Exclusion Chromatography (HPSEC) system consisted of a Waters 1515 Isocratic pump with a differential refractive index detector (Waters 2414). The mobile phase (0.1 M phosphate buffer, pH 6.2) was eluted through a Waters Ultrahydrogel 1000 column with a flow rate of 0.5 mL min^{-1} where the column temperature was kept constant at 37°C . ChromManager software was used to integrate the chromatograph peaks. The HPSEC system was calibrated using dextran standards (as above). The standards (2 mg mL^{-1}) were prepared fresh daily by dissolving in distilled water within 10 mL screw tubes after immersing into a boiling water bath for 10 min. Upon cooling, the tube contents were filtered ($0.20 \mu\text{m}$, Sartorius Minisart 16532 syringe filters) then injected into the HPSEC $20 \mu\text{L}$ injection loop (in order of decreasing molecular weight). Solutions of β -limit dextrin (BLD) (10 mg mL^{-1}) were prepared by an additional step which involved

^{*} Corresponding author. Tel.: +44 141 3318618.

E-mail address: k.alvani@glycologic.co.uk (K. Alvani).

Table 1

Number (M_n) and weight (M_w) average molecular weight of dextrans and β -limit dextrans.

Sample	\bar{M}_n	\bar{M}_n^c	\bar{M}_w
<i>Dextran (standards)</i>			
25k-Fluka-31419	1.83×10^{4a}	2.80×10^4	2.38×10^{4a}
80k-Fluka-31421	5.55×10^{4a}	8.01×10^4	8.09×10^{4a}
100k-Fluka-09184	–	1.12×10^5	–
150k-Fluka-31422	1.00×10^{5a}	1.50×10^5	1.48×10^{5a}
270k-Fluka-31423	1.64×10^{5a}	2.69×10^5	2.73×10^{5a}
410k-Fluka-31424	2.36×10^{5a}	4.08×10^5	4.10×10^{5a}
500k-Fluka-31392	–	5.10×10^5	–
<i>BLDs</i>			
BLD194	4.80×10^{5b}	2.09×10^5	5.60×10^{6b}
BLD196	5.90×10^{5b}	2.40×10^5	5.60×10^{6b}
BLD197	2.80×10^{5b}	2.63×10^5	3.70×10^{6b}

^a Fluka (by gel permeation chromatography (GPC)).

^b HPSEC.

^c Reducing method.

initially dissolving the BLDs in dimethyl sulphoxide (DMSO, 90% v/v) within 10 mL screw cap tubes. The tubes were placed into a boiling water bath to facilitate dissolving followed by the addition of small magnetic bars and constant stirring overnight to remove any gel. Aliquots (1 mL) of the BLD solutions were transferred by pipette into clean 10 mL tubes followed by precipitation with 9 mL cool ethanol (80%, v/v). Next, the tubes were centrifuged (2500g for 10 min, 4 °C), and the procedure was repeated three times, to remove impurities (including maltose) (Kajiwarra & Maeda, 1983; Ku, Jansen, Oles, Lazar, & Rader, 2003). The precipitant were then re-dissolved in distilled water (5 mL) in a boiling water bath (10 min) combined with magnetic stirring as above. The samples were cooled to room temperature, filtered (0.20 μ L, Sartorius Minisart 16532) then injected (via 0.20 μ m loop) into the HPSEC system.

2.3. Reducing power method

The carbohydrate reducing power was obtained by a modified Nelson's method in triplicate as described below.

Reagents: Stock solutions of dextrans (Table 1) or β -limit dextrin (BLD) (25 mg mL⁻¹) were prepared as above.

Nelson's Reagents (Arsenomolybdate, 'A' and 'B' and Nelson's A + B Reagent) were prepared according to the standard Nelson's method (Nelson, 1944).

Reference (blank) solutions were prepared by transferring 0.4 mL of DMSO solution (90%) into clean 10 mL screw cap tubes to which 0.6 mL distilled water was added (with mixing).

A standard glucose solution for calibration (100 μ g mL⁻¹) was prepared by dissolving 100 mg pure anhydrous glucose in 1 L DMSO solution (90%) within a 1 L flask.

Aliquots (1 mL) of dextran standards or BLD stock solutions were transferred by pipette into clean 10 mL screw cap tubes. These were precipitated with 9 mL cool ethanol (80%, v/v). After sealing, the tubes were mixed by gentle shaking, centrifuged (2500g for 10 min, 4 °C) which was repeated three times to remove impurities (including maltose). The precipitates were then re-dissolved in 90% DMSO (1 mL) by placing the capped tubes in a boiling water bath for 10 min followed by the addition of small magnetic stirrers with mixing to facilitate dissolution. Aliquots (0.4 mL) of the BLD-DMSO solution were transferred by pipette into clean 10 mL screw cap tubes to which 0.6 mL distilled water was added. Nelson's reagent (A + B) (1 mL) was added to all tubes (reference, standard, dextran and BLD samples, each 1 mL) by pipette. After mixing, the tubes were sealed, placed in a boiling water-bath for

exactly 20 min after which the samples were cooled to room temperature (cold water for 5 min) then mixed again thoroughly. To each tube, 1 mL of Nelson's Arsenomolybdate Reagent (from a dispenser) was then added, the tubes were sealed and vortex mixed. Next, 7 mL distilled water (by dispenser) was added to each tube. All tubes were sealed and mixed again. The absorbance was read on a spectrophotometer at 500 nm. The absorbance of the reference tubes was subtracted from the glucose and dextran standards plus the BLD samples. The \bar{M}_n was calculated as follows:

$$\bar{M}_n = \frac{\alpha\text{-glucan} \times (A_G - A_R) \times 162 (M_w \text{ polymerised glucose})}{(A_S - A_R) \times 0.1}$$

where A_G = absorbance of the glucose standard; A_R = absorbance of the blank; and A_S = absorbance of sample.

3. Results and discussion

Table 1 shows the number (\bar{M}_n) and weight average molecular weight (\bar{M}_w) of dextrans and BLD samples as determined by Fluka (dextrans only), by HPSEC and the modified (Nelson, 1944) reducing method. Using the reducing method, the \bar{M}_n of the dextran standards was very similar to the data provided by Fluka. The \bar{M}_w figures reported by Fluka for the dextran standards were always (and perhaps unsurprisingly) higher than the corresponding \bar{M}_n data. For the BLD samples, calculating \bar{M}_n from the retention time (volume) by HPSEC (Table 1) indicates that \bar{M}_n ranges from 2.80 – 5.90×10^5 . The corresponding figures by the reducing method ranged from 2.09 – 2.63×10^5 . Hence, even when taking into account the polydispersity of the BLD polysaccharides, the two techniques provided broadly similar results. With reference to M_w (HPSEC), the molecular weight was higher and more similar to the M_w s reported typically for BLDs (Ammeraal & Friedman, 1996) It is recognised that the M_w for the BLD samples represents an extrapolation in MW from the dextran standards and that the dextrans and dextrans may not elute exactly the same way (due to differences in solvent and column interactions). However, this does not detract from the overall similarity between \bar{M}_n determined by HPSEC and the reducing method.

4. Conclusions

A method has been developed based on the reducing power of dextrans and dextrans to rapidly estimate \bar{M}_n (without any reference to polydispersity) without the need to employ chromatographic (e.g. HPSEC) techniques. The method provides data similar to those generated by HPLC and could be used where chromatographic systems are not available.

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