On the molecular weight distribution of dextran T-500

ABIGAIL BALL, STEPHEN E.HARDING and NEIL J.SIMPKIN
University of Nottingham, Department of Applied Biochemistry & Food Science, Sutton Bonington LE12 5RD, UK

ABSTRACT

The combined size exclusion chromatography/ low speed sedimentation equilibrium method is used to obtain an absolute molecular weight distribution for a commercially used dextran (T-500, Pharmacia). The form of the distribution and the weight average molecular weight for unfractionated T-500 [$(0.50\pm.02) \times 10^5$] are in good agreement with observations from, for example, on-line size exclusion chromatography/ multi-angle laser light scattering.

INTRODUCTION

One of the most important uses of dextrans is in medicine, for thickening blood plasma. For the food industry dextrans are mainly used as standards for analytical purposes for monitoring the performance (and in some cases calibrating) chromatographic separation processes. One of the most commonly used "standards" is dextran T-500 (so called because it has a molecular weight of ~500kD). In this short study we determine the molecular weight distribution of dextran T-500 by using a combined approach involving size exclusion chromatography (SEC) and low speed sedimentation equilibrium (LSSE) in the analytical ultracentrifuge.

MATERIALS

Dextran T-500 was supplied commercially from Pharmacia (Milton Keynes, U.K.) The solvent used for all analyses was a standard phosphate chloride buffer (pH 6.5, I 0.30). The relevant proportions of Na_2HPO_4 and KH_2PO_4 were made up to a combined ionic strength of 0.05. A total ionic strength of 0.30 was achieved by adding relevant proportions of NaCl in accordance with Green (1).

METHODS

Size exclusion chromatography

A column of Sephacryl S-400 (Pharmacia) was used, of 1.6cm internal diameter and 73cm height. Loading concentrations were ~ 1mg/ml, and sample volumes were 5ml. The flow rate was 10ml/hour.

Fractions of 2ml were collected and assayed for total sugar content using a phenol sulphuric acid procedure similar to that described by Dubois et al (2). Elution volumes were determined by weight. The void volume was determined using blue dextran 2000 and the total volume using sucrose. Column recoveries were between 90 and 100%.

Sedimentation Equilibrium

The low speed method (see ref. 3) was used in a Beckman Model E analytical ultracentrifuge equipped with an RTIC temperature measuring system, Rayleigh interference optics and a 5 mW He-Ne laser light source using procedures described by Creeth & Harding (4). All determinations were made in 30mm optical path length cells at the lowest possible loading concentrations (0.1-0.5 mg/ml) to minimise possible effects of thermodynamic non-ideality and associative phenomena. At the concentrations used, non-ideality effects are likely to contribute less than ~ 5% error in the measurement (see Table 2.2 of ref 5) which is of the same order as the precision of the measurement. A value of 0.613 ml/g was used for the partial specific volume (6).

Molecular weight determinations (apparent weight averages, M_w) of the fractions were mainly performed in groups of three using an Yphantis-style multi-channel 30mm path length cell. All samples had been made up in the solvent as described above and dialysed against this solvent. Whole cell weight average molecular weights were extracted using the M* function as described by Creeth & Harding (4).

Calibration of the SEC column using low speed sedimentation equilibrium

Fractions of 2ml volume were isolated from the eluate, and fractions of equal elution volume from a series of three or four runs were combined and concentrated using centrisart tubes (Sartorious Ltd.), to a concentration of ~0.2mg/ml. The molecular weights of these fractions were then determined by LSSE, as described, and a plot of the logarithm of the (apparent) weight average molecular weight, M_w versus elution volume obtained. The calibration plot and the elution profile were then used to determine the molecular weight distribution. This is the method described by Ball et al (7).

RESULTS

Molecular weight distribution

The plots of elution volume, calibration and molecular weight distribution are given in Figure 1 (a,b,c respectively).

A linear calibration appeared reasonable (Figure 1b) within

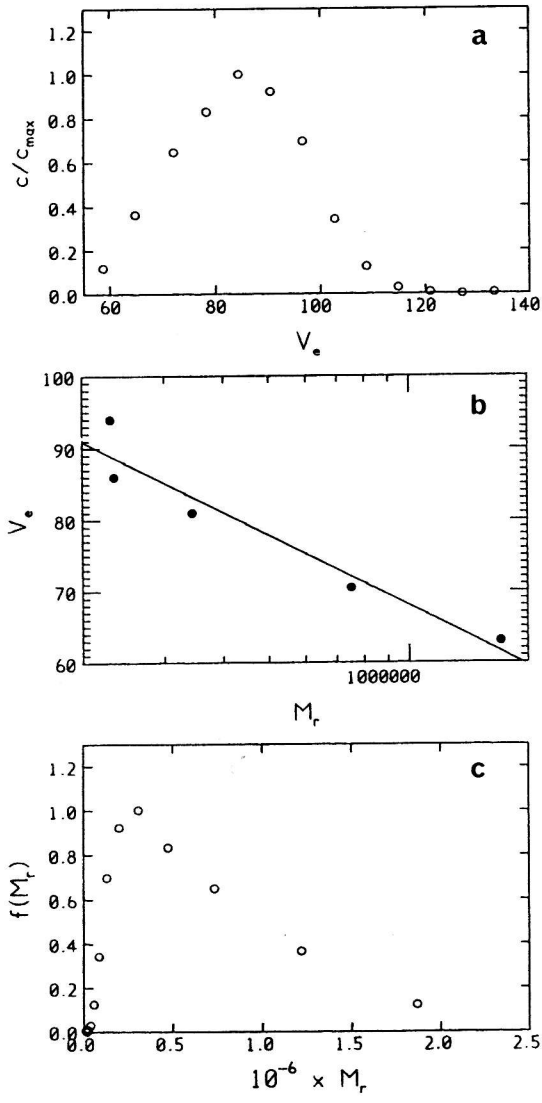

Figure 1. Calibrated Gel Chromatography for Dextran T-500. (a) Elution profile; (b) Calibration plot from low speed sedimentation equilibrium; (c) Molecular weight distribution

the range of molecular weights examined. The distribution is of a log-normal type with a mode molecular weight of $\sim 3 \times 10^6$. Molecular species with an M_w as high as 2.0×10^6 were present. From the form of this distribution, an approximate value for the weight average over the whole distribution of $\sim 0.4 \times 10^6$ was obtained.

Weight average molecular weight of unfractionated material

LnJ vs ξ plots, [where J is the absolute concentration in fringe number units and ξ is a radial displacement squared parameter] were appreciably more upward curved for unfractionated than fractionated material - corresponding to the greater heterogeneity. A weight average molecular weight over the whole distribution for the unfractionated material of $(.50 \pm .02) \times 10^6$ was obtained.

CONCLUSIONS

The molecular weight of 500kD quoted by the commercial manufacturer for dextran T-500 was confirmed. Polydispersity was however rather high with species of M_w as high as $\sim 2.0 \times 10^6$ present. The data for the average molecular weight and the distribution are in reasonable agreement with independent measurements using on-line SEC/ multi-angle laser light scattering (8), and also earlier sedimentation equilibrium studies (9).

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