Note

Molecular characterization of fructans by high-performance gel chromatography

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Linear β-2,1-linked fructans (inulins) occur as reserve carbohydrates in Compositae and Campanulaceae[1]. Fructans with a linear β-2,1 backbone, branched β-2,6, are deposed by certain Liliaceae in their perennial rhizomes[2]. As is well known from the glucan series, it might be expected that these two different fructan types would differ considerably in their physico-chemical behaviour. The linear inulin, assumed to have a helical structure[3], is soluble only in hot water and can be crystallized from it by cooling or precipitation with alcohol. The highly branched Liliaceae fructans, in this work commercially available sinistrin from red squill and a laboratory-prepared fructan from *Lilium bulbiferum*, are amorphous, glassy and hygroscopic substances that are very soluble in cold water and cannot be precipitated with even 80% methanol.

The molecular characterization of fructans by modern chromatographic methods has been achieved using either high-performance liquid chromatography (HPLC)[4,5] or low-pressure gel permeation chromatography (GPC)[6-8]. The more time-consuming GPC has the advantage that the average molecular weights $\overline{MW}_w$ and $\overline{MW}_n$, the average chain lengths $\bar{P}_w$ and $\bar{P}_n$ and the dispersity factor $\bar{P}_w/\bar{P}_n$ can be calculated easily using the integral mass-distribution function $I(P)$[6]. The HPLC methods offer the possibility of determining the concentration of single sugar oligomers, but the molecular parameters that are necessary for characterizing the disperse distribution of polymers cannot be calculated.

A high-performance GPC method is described that reduces the inconveniently long analysis time of low-pressure GPC to that of the silica HPLC method. Similarly to low-pressure GPC, this new method allows the molecular parameters of fructans to be calculated after appropriate calibration.

EXPERIMENTAL

Commercially available inulin (chickory) and sinistrin (red squill) were gifts from the Laevosan Gesellschaft (Linz, Austria). Native and purified inulin from Jerusalem artichoke tubers and chickory roots were isolated as described earlier[6]. Fructan from *Lilium bulbiferum* was prepared analogously to the inulins.

A 20-μl volume of a 5% fructan solution was applied with a Rheodyne 7125

loop valve to a Superose 12 column (Pharmacia) (300 × 10 mm I.D.); solvent deaerated distilled water was used (LKB 2150 pump). Detection was effected with a Waters Assoc. RI 401 detector and monitoring with an LKB 2210 flat-bed recorder. The separation was carried out at room temperature. Calculation and calibration of the chromatographic system were carried out analogously to low-pressure GPC as described by Praznik and Beck[6]. The void volume was determined with dextran 5000 (a gift from R. W. Klingler). The total volume of the system was determined with deuterium oxide (Merck, Darmstadt, F.R.G.).

RESULTS AND DISCUSSION

It is well known that the migration behaviour in GPC is influenced by the structure of polysaccharides. It has been shown that the higher the molecular weight of a polysaccharide, the more its migration behaviour differs[9]. In the low-molecular-weight region of polysaccharides (less than 20 000 daltons) the structure was found not to influence the migration behaviour significantly on low-pressure gel columns. A narrow packed, high-pressure packing material, however, is not only expected to act as a simple gel matrix as described above, but also, because of the counter-pressure and pressure decrease in the column, the migration behaviour might be influenced by the intrinsic viscosity of samples[10]. Shearing and other effects should also not be neglected in this context. If this hypothesis were true, a slight difference in the intrinsic viscosity of the samples would distinctly affect the migration behaviour in high-pressure GPC systems.

As can be seen from Fig. 1, the calibration graph of dextrans differs significantly from that of the fructans. This indicates that the structural construction of the monosaccharide subunits and the different polymeric structure, resulting in a different intrinsic viscosity $[\eta]$ and therefore in a different hydrodynamic volume $[\eta]$ · MW, affects the migration behaviour on narrow packed gel columns even in a relatively low-molecular-weight region[10].
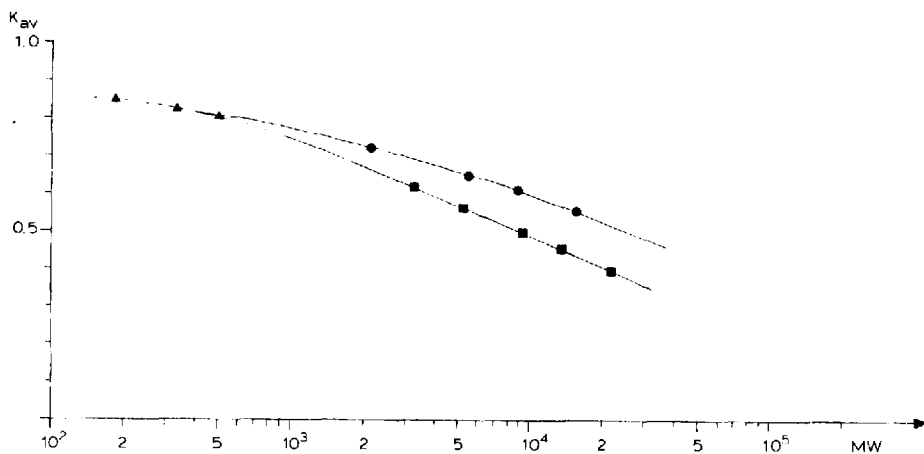

Fig. 1. Calibration graph for the Superose 12 column: relationship between the distribution coefficient, K_{av}, and the molecular weight. ■, Dextran T 10, T 40; ▲, fructose, sucrose, isokestose; ●, molecular defined (osmometry, light scattering) inulins.

Figs. 2 and 3 show the separation of linear and branched fructans. Possible impurities such as proteins or pectins occurring in biological materials are totally separated from the fructans and do not disturb the calculation. This means that the samples need not be highly purified before being applied to the column, which is desirable in low-pressure GPC[6] and necessary in HPLC[4]. This is especially important for fructans, because of their acid lability. Even on passing through an acidic ion exchanger hydrolysis can occur.

The purified fructans are narrowly distributed, as expected. The native fructans, however, have a dispersity $\bar{P}_w/\bar{P}_n \approx 2.0$, which corresponds well with the idea that linear unbranched polysaccharides should be binomially distributed[11]. Table I lists the molecular parameters of the fructans shown in Figs. 2 and 3.

It is remarkable that the branched fructans have a dispersity factor under 1.40. Especially the native fructan from *Lilium bulbiferum* is remarkably uniform compared with the native inulins. This uniformity could lead to the notion that some "repeating
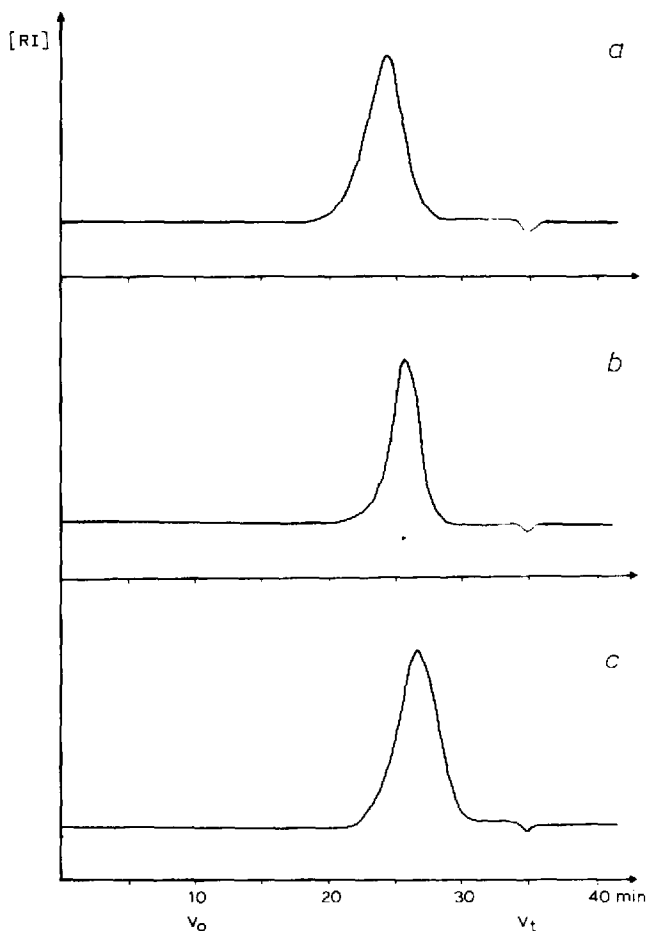

Fig. 2. High-performance GPC separation on Superose 12 of purified (a) Jerusalem artichoke inulin, (b) chickory inulin and (c) sinistrin (red squill).
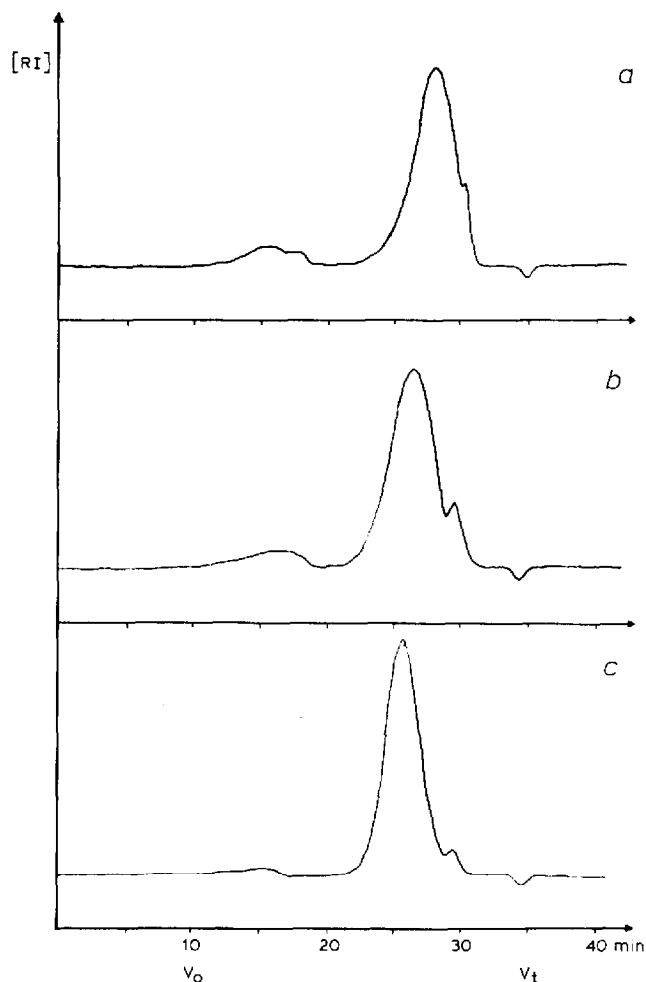
Fig. 3. High-performance GPC separation on Superose 12 of native (a) Jerusalem artichoke inulin, (b) chickory inulin and (c) fructan from *Lilium bulbiferum*.

TABLE I

NUMBER- AND WEIGHT-AVERAGE MOLECULAR WEIGHTS AND CHAIN LENGTHS AND DISPERSITY FACTORS OF CERTAIN FRUCTANS

State	Fructan	$\overline{MW}_w$	$\overline{MW}_n$	$\bar{P}_w$	$\bar{P}_n$	$\bar{P}_w/\bar{P}_n$
Purified	Inulin (Jerusalem artichoke)	10 500	8800	65	54	1.20
	Inulin (chickory)	6450	5300	40	33	1.22
	Sinistrin (red squill)	7000	5070	43	31	1.38
Native	Inulin (Jerusalem artichoke)	2900	1300	18	8	2.22
	Inulin (chickory)	4450	2350	28	15	1.88
	Fructan (*Lilium bulbiferum*)	3180	2430	20	15	1.31

blocks" of oligosaccharide sub-chains are used for the synthesis of this polysaccharide.

REFERENCES

1 C. J. Pollard and K. S. Amuti, *Biochem. Syst. Ecol.*, 9 (1980) 69.
2 C. L. Pollard, *Biochem. Syst. Ecol.*, 10 (1982) 245.
3 J. R. Katz and J. C. Derksen, *Recl. Trav. Chim. Pays-Bas*, 50 (1930) 248.
4 W. Praznik, R. H. F. Beck and E. Nitsch, *J. Chromatogr.*, 303 (1984) 417.
5 Y. Mino, S. Tsutsui and N. Ota, *Chem. Pharm. Bull.*, 33 (1985) 3503.
6 W. Praznik and R. H. F. Beck, *J. Chromatogr.*, 348 (1985) 187.
7 C. J. Pollock, M. A. Hall and D. P. Roberts, *J. Chromatogr.*, 171 (1979) 411.
8 Ch. Labhart, J. Nösberger and C. J. Nelson, *J. Exp. Bot.*, 34 (1983) 1037.
9 W. Praznik, *Starch*, in press.
10 T. Kuge, K. Kobayashi, H. Tanahasahi, T. Igushi and S. Kitamura, *Agric. Biol. Chem.*, 48 (1984) 2375.
11 E. Husemann and B. Pfannemüller, *Makromol. Chem.*, 87 (1965) 139.