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Globular shape of high molar mass inulin revealed by static light scattering and viscometry

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Abstract

The behavior of two high molar mass inulin-type fructans in dilute aqueous and dimethyl sulfoxide solution was investigated. We performed static light scattering experiments (SLS) and viscometry with the polymer synthesized in vitro using the fructosyltransferase (FTF) of *Streptococcus mutans* that was expressed as a fusion protein in transgenic *Escherichia coli*. In addition, high molar mass inulin of *Aspergillus sydowi* (*A. sydowi*) was synthesized by incubating conidia with highly concentrated sucrose solution. This polymer was characterized by SLS and high-performance size-exclusion chromatography.

All samples showed small root-mean-square radii of gyration with respect to their very high molar mass. Since this suggests a compact conformation of the molecules in both solvents, the dependence of reduced osmotic modulus M_w/M_{app} on parameter $X = A_2 M_w c$ was studied by SLS. For all measurements, we found an agreement between experimental values and theoretical curves for hard spheres. Hydrodynamic data also point to a globular shape of high molar mass inulin in dilute solution.

The determination of branches for inulin synthesized by FTF and by *A. sydowi* conidia indicates that both polymers are β-(2 → 1) linked polyfructans with 5–7% β-(2 → 6) branches, which is in agreement with the obtained globular molecular shape in dilute solution.

Keywords: Inulin; Light scattering; Viscometry

1. Introduction

Fructans are oligomeric or polymeric carbohydrates that are synthesized from sucrose and consist of a fructose chain, which may contain a terminal glucose molecule. Fructan synthesis is widespread among bacteria, occurring in gram-positive as well as gram-negative families, and has also been demonstrated for some fungal species [1]. Fructans synthesized by bacteria as a component of the exopolysaccharide are high molar mass polymers, which are in almost all cases of the levan type, characterized by the β-(2 → 6) linkage type of fructose monomers [2]. The only bacterial species known so far that produces an inulin-type fructan consisting of β-(2 → 1) linked fructose molecules is *Streptococcus mutans* (*S. mutans*) [3]. This polysaccharide has a molar mass of 20×10^6 g/mol and contains not more than 5% β-(2 → 6) linked branches [4,5].

In a recent paper [6], we have described the construction, purification and characterization of a fusion protein of maltose-binding protein of *Escherichia coli* and the fructosyltransferase (FTF) of *S. mutans*. With the purified protein, we have performed in vitro synthesis of high molar mass inulin (FTF-inulin) and the enzymatic properties of the fusion protein were studied. High-performance size exclusion chromatography (HPSEC) and static light scattering (SLS) measurements were carried out to characterize the obtained inulin. For the in vitro synthesized FTF-inulin samples, very high weight average molar masses between 50×10^6 and 90×10^6 g/mol and a remarkable small polydispersity index of 1.1 have been determined. The values of the root-mean-square radii of gyration of 40–50 nm for inulin in water and 50–80 nm in dimethyl sulfoxide (DMSO) are small with respect to the high molar mass of

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this polymer. Consequently, the macromolecules were assumed to show a compact globular shape in dilute solution of both solvents.

Whereas high molar mass levan has been reported to be a highly branched polymer that shows a compact structure in water [7] and DMSO [8], no references have been found examining inulin samples with a molar mass of up to 90×10^6 g/mol. Only a study of inulin in a broad range of molar mass from 14 900 to 5×10^6 g/mol has been published by Kitamura et al. [9], but these polymers were synthesized from sucrose by *A. sydowi* conidia [10,11]. According to the authors, this inulin was assumed to be essentially linear.

In the present paper, we report on the investigation of the shape of enzymatically synthesized high molar mass FTF-inulin in dilute solution by means of light scattering measurements. To understand the solution behavior in more detail, viscometry was performed to discuss the relationship between molar mass and intrinsic viscosity.

In this study we also describe the characterization of inulin synthesized by *A. sydowi* conidia (*A. sydowi*-inulin) by means of SLS and HPSEC in dilute solution. The re-investigation of *A. sydowi*-inulin was aimed at comparing the degree of branching of both inulin polymers (FTF-inulin and *A. sydowi*-inulin).

Whereas linear macromolecules should exhibit a coil conformation, for a branched polymer, a globular shape in dilute solutions is expected.

2. Experimental

2.1. Polymer samples

FTF-inulin was synthesized by the in vitro method reported recently [6].

A. sydowi-inulin was prepared as follows: *Aspergillus sydowi* IAM 2544 was obtained from the Institute of Applied Microbiology, Tokyo, University of Tokyo, Japan.

The fungus was grown on solid medium containing 2% malt extract, 0.5% peptone (Difco Laboratories, Detroit MI, USA)), 2% sucrose and 1.5% agar and conidia were harvested after 12 days incubation at 30°C. Contaminating mycelia were removed by filtration through cotton gauze. The conidia were collected by filtration through MF-Millipore filter (pore size 0.45 μm).

Conidia were incubated in 10 mM sodium phosphate buffer, pH 5.5, containing 20% sucrose and 20 mM L-cystein for 3 days at 30°C as described by Harada et al. [11]. After incubation, conidia were removed by filtration through MF-Millipore filter (pore size 0.45 μm), and the inulin in the filtrate was precipitated by adding 2 volumes of ethanol. To obtain a pure high molar mass inulin, the precipitate was dissolved in water, dialyzed two times against a 100-fold excess of water using dialysis tubing with a molar mass cut-off of 15 000 g/mol and again precipitated with 2 volumes of ethanol.

2.2. Measurements

2.2.1. High-performance size-exclusion chromatography

The molar mass distribution and the root-mean-square radius of gyration (R_g) of the inulin samples were determined by means of two different HPSEC systems (aqueous salt solution and DMSO as eluent).

Aqueous eluent: The HPSEC system consisted of a Waters 150CV integrated SEC device with pump, autoinjector, column compartment, differential refractive index detector (DRI) and multi-angle laser light scattering (MALLS) detector. The MALLS detector, a Dawn-F-DSP laser photometer (Wyatt Technology, Santa Barbara), was equipped with a K5 flow cell, with a He–Ne laser operating at $\lambda_0 = 632$ nm and with 18 detectors at angles ranging from 14.4 to 163°. A set of three SUPREMA columns (SUPREMA 30000, 1000 and 100) from PSS, Mainz with a dimension of 300×8 mm^2 were used in series. The elution was carried out with 0.05 M aq. $NaNO_3$ at a flow rate of 0.735 ml/min and a temperature of 75°C.

DMSO eluent: The HPSEC system (Waters) consisted of a 600MS pump module, 717 autoinjector, column compartment, RI-detector 410 and MALLS detector, a Dawn-F-DSP laser photometer (Wyatt Technology, Santa Barbara) fitted with a S2 flow cell and an Ar-ion laser operating at $\lambda_0 =$ 488 nm and equipped with 18 detectors at angles ranging from 7.5 to 157°. The columns were Waters Styragel HMW 7, HMW 6E and HT 3 with a dimension of 300×7.8 mm^2. The elution of samples was carried out with DMSO containing 0.09 M $NaNO_3$ at a flow rate of 0.5 ml/min and a temperature of 60°C.

2.2.2. Static light scattering

The weight average molar mass (M_w) and the root-mean-square radius of gyration (R_g) were determined by SLS. The measurements were carried out in dilute aqueous and DMSO solution at 33 and 25°C, respectively, using a modified (SLS-Systemtechnik, Hausen i.W., Germany) computer-controlled Fica 50 (Fica, Le Mesnil-Saint Denis, France) equipped with a laser operating at $\lambda_0 = 543.5$ nm. The intensity of scattered light was detected with a photomultiplier at angles ranging from 45 to 145°. The solutions were made dust-free by centrifugation at about 3000*g* for 15 min. The refractive index increments were determined with a differential refractometer according to Asmussen and Springer [12] at 546 nm (inulin/water at 33°C: 0.1313 cm^3/g; inulin/DMSO at 25°C: 0.0659 cm^3/g).

The general procedure for evaluation of the SLS data has been described elsewhere [6].

2.2.3. Viscometry

The solution viscosity of FTF-inulin was measured with a rotational viscometer CV 100 (Haake, Karlsruhe, Germany) of the Couette type, using cup and bob geometry (ZB 30) with inner and outer radii of 29.36 and 30.00 mm,

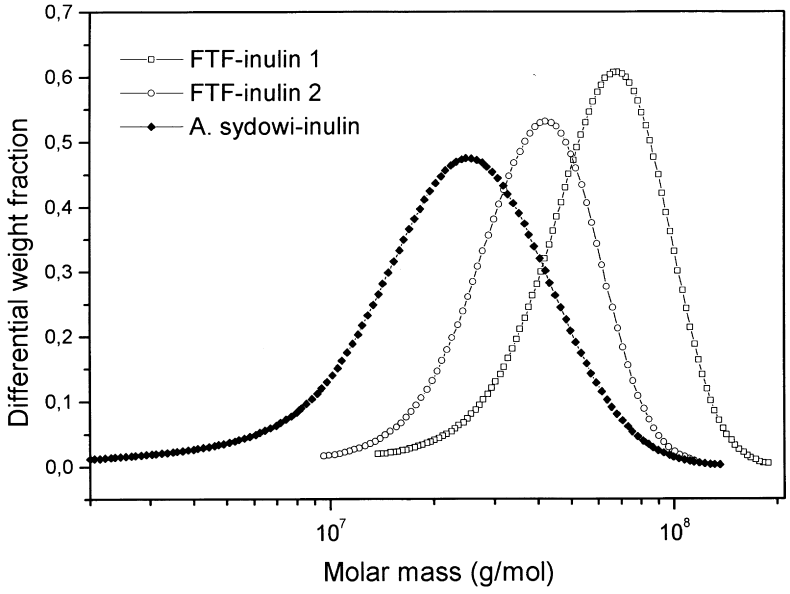

Fig. 1. Molar mass distribution of *A. sydowi*-inulin obtained by HPSEC, for comparison also two FTF-inulin samples are shown.

respectively. The shear rate was increased from 0 to 1000 l/s at 20°C both in DMSO and in water.

Solution concentration, determined by dry weight measurements, was varied between 0.5 and 1.2 g/l for DMSO and 0.5 and 1.6 g/l for water. Inulin is soluble in DMSO at ambient temperatures, whereas the aqueous solution of inulin had to be heated at 60°C for 15 min. Both solutions were agitated for 24 h at room temperature (r.t.) before performing the measurements.

The intrinsic viscosity $[\eta]$ of an FTF-inulin sample of $M_w = 57 \times 10^6$ g/mol was measured in deionized water at 25°C using an Ubbelohde type capillary viscometer (capillary type 53103-Oc, Schott, Mainz, Germany). The solution concentration ranged from 1.2 to 8 g/l. The flow time of the solvent was 345 s. Kinetic energy correction was always neglected.

2.3. Determination of branching

2.3.1. Methylation analysis of inulin samples

Permethylation was carried out according to the method of Ciucanu and Kerek [13] and is described for example for *A. sydowi*-inulin. This inulin (24.6 mg, corresponding to 0.456 mmol OH) was dissolved in dry DMSO (2.5 ml) under nitrogen. Freshly prepared pulverized sodium hydroxide (61 mg, 1.52 mmol, 3.3 equiv./OH) was added. After 20 min. stirring at room temperature MeI (100 μl, 1.62 mmol, 3.5 equiv./OH) was added and the suspension was stirred overnight at r.t. The reaction mixture was poured into water and extracted with dichloromethane two times. The organic extracts were washed with water four times and then dried over calcium chloride. After evaporation of the solvent, the methylated inulin was obtained as a colorless clear film (25.1 mg, yield 81%). Portions of about 2 mg of the permethylated inulin were submitted to acid hydrolysis with 0.5 M trifluoroacetic acid at 100°C for 1 h. After careful evaporation of the acid and codistillation with toluene to remove traces of acid, reduction was performed with sodium borohydride-d_4 in 0.5 M ammonia (0.5 M, 0.5 ml) for 1 h at 60°C. Excess of the reagent was destroyed with acetic acid, and then the borate was removed by evaporation with MeOH/acetic acid for five times. The residue was acetylated with acetic anhydride (150 μl) and pyridine (25 μl) at 90°C for 2.5 h. The acetic anhydride was destroyed with $NaHCO_3$ solution, the products extracted with dichloromethane and thoroughly washed with aq. $NaHCO_3$ and subsequently with water. The dried organic phase was used for gas–liquid chromatography (GLC) and gas–liquid chromatography–mass-spectrometry (GLC–MS) analysis.

2.3.2. Reductive cleavage

Reductive cleavage was performed according to Rolf and Gray [14]. The permethylated inulin (2.0 mg) was dissolved in dichloromethane (0.025 mmol solution). Triethylsilane (14.1 μl, 9 equiv.) and $Me_3SiOSO_2CF_3$ (7.8 μl, 4.4 equiv.) was added. To the reaction mixture acetic anhydride (15 μl) was added after 2.5 h or 5 h at r.t.. After further 2 h, the reaction was quenched with $NaHCO_3$ solution. The organic phase was washed with aq. $NaHCO_3$ and water, dried and used for GLC and GLC–MS analysis. A second reaction was performed with 5 equiv. of each silane and Lewis acid for 2 h at room temperature.

GLC and GLC–MS were performed as described in Ref. [15].

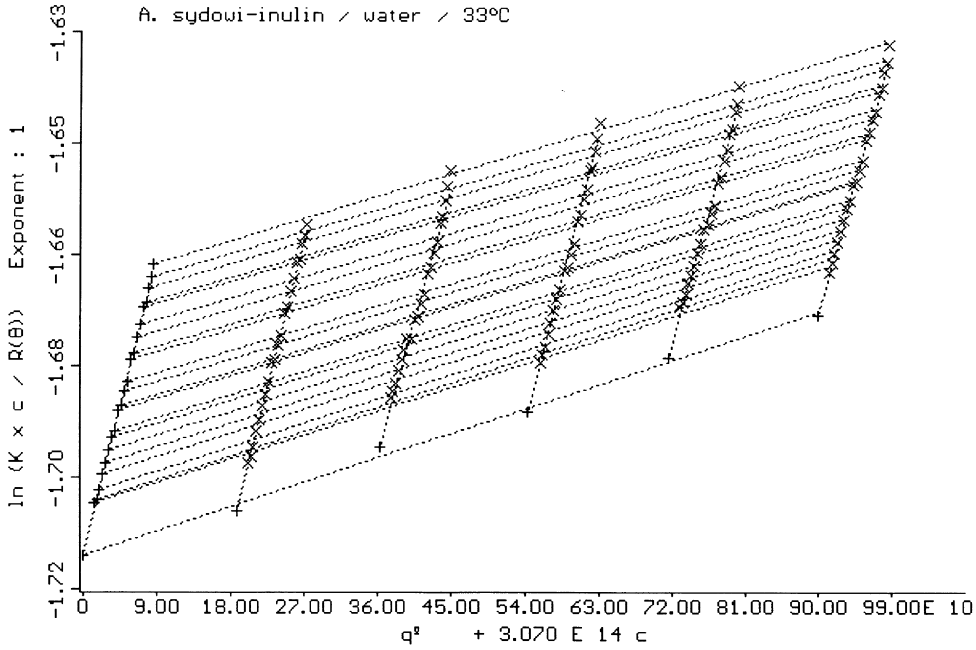

Fig. 2. Guinier plot of *A. sydowi*-inulin in water at 33°C. Concentrations: 0.6111 g/l; 1.1815 g/l; 1.7733 g/l; 2.3331 g/l; 2.9292 g/l.

3. Results and discussion

3.1. Static light scattering and high-performance size-exclusion chromatography

Static light scattering and high-performance size-exclusion chromatography were performed to determine weight-average molar mass, molar mass distribution and radius of gyration of the polymers. The results for FTF-inulin were reported recently [6]. For *A. sydowi*-inulin and two higher molar mass FTF-inulin samples the molar mass distribution obtained by HPSEC in DMSO at 60°C is displayed in Fig. 1.

SLS measurements of the same *A. sydowi*-inulin polymer were carried out in water at 33°C. From the Guinier plot (Fig. 2), a weight-average molar mass of 28×10^6 g/mol and a value of the *z*-average root-mean-square radius of gyration of 40 nm was observed.

Table 1
Results of molecular characterization of inulin samples in water and DMSO by HPSEC/SLS, M_w: weight-average molar mass, R_g: *z*-average root-mean-square radius of gyration and M_w/M_n: polydispersity index

Sample	Water		DMSO		M_w/M_n
	M_w HPSEC/SLS	R_g	M_w HPSEC/SLS	R_g	
	(10^6 g/mol)	(nm)	(10^6 g/mol)	(nm)	
FTF-inulin 1	71/71	48/47	70/68	64/55	1.1
FTF-inulin 2	48/–	42/–	44/–	60/–	1.1
FTF-inulin 3	33/–	42/–	–/–	–/–	1.1
A. sydowi-inulin	26/28	39/40	27/–	62/–	1.7

As expected, with SLS and HPSEC the same M_w were determined for the polyfructans independent of solvent and temperature (see Table 1). For *A. sydowi*-inulin, a somewhat higher polydispersity index was observed. The values of the *z*-average root-mean-square radius of gyration in aqueous solution match for both methods. However, for the system inulin/DMSO, a much higher R_g value was obtained by HPSEC. Taking into account that the SEC measurements were performed at higher temperatures compared to SLS investigations, it is assumed that the thermodynamic quality of the solution does not change with increasing temperature in the system inulin/water but does so in the system inulin/DMSO.

From the HPSEC measurements it is possible to generate a log–log plot of R_g as a function of M_w (see Fig. 3). From the slope of such a plot, the molecular conformation can be derived. Theoretical slopes of 0.33, 0.50 and 1.0 are expected for spheres, random coils in theta solvents and rigid rods, respectively. For most real random coils, slopes in the range of 0.55–0.60 have been reported [16]. As can be seen from Fig. 3 for FTF-inulin, a linear fit can be performed. The values of exponent of 0.26 and 0.28 for FTF-inulin indicate a compact conformation in both solvents, as shown for example in water.

For *A. sydowi*-inulin, however, the slope is not constant over the investigated molar mass range of the sample. Therefore no slope value could be determined. Since the slope of the log–log conformation plot is also an indicator of branching, from the shape of the curve for *A. sydowi*-inulin, a dependence of branching on molar mass could be concluded. At smaller molar masses FTF-inulin and *A. sydowi*-inulin show approximately the same slope, which

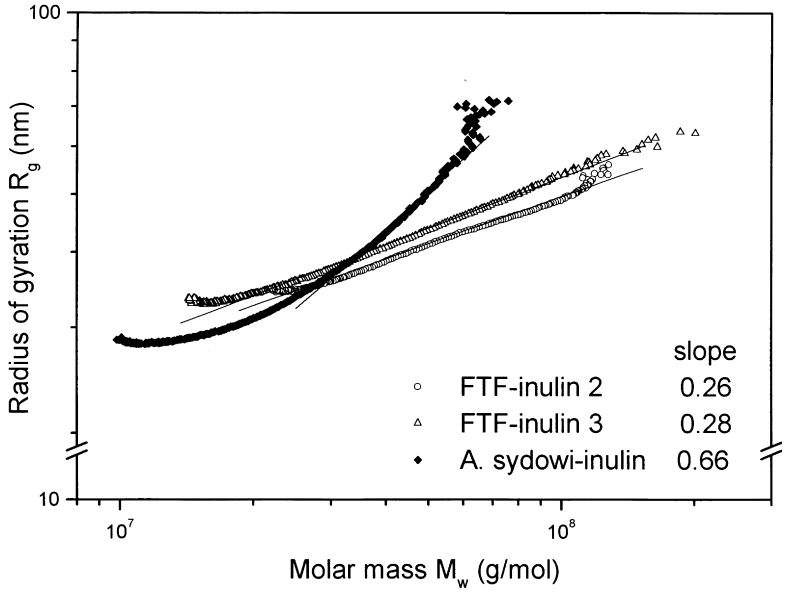

Fig. 3. Root-mean-square radius of gyration as function of weight-average molar mass (conformation plot, lines: linear fit curves).

points to similar conformation. The much larger slope for *A. sydowi*-inulin at higher molar masses indicates a less compact structure in comparison to FTF-inulins with the same molar mass. From the described behavior also, a different type or amount of branching could be assumed. Therefore the determination of branchpoints was performed for FTF-inulin and *A. sydowi*-inulin samples of the same molar mass of 30×10^6 g/mol (see Section 3.3).

Another possibility to characterize the conformation of macromolecules in dilute solution is the analysis of SLS measurements. The intensity of scattered light extrapolated to scattering vector $q \rightarrow 0$ gives the inverse osmotic compressibility $(1/RT)(\partial\pi/\partial c)_T$ [17], which is usually denoted as osmotic modulus or as structure factor at zero angle. Following the theory, for different macromolecular architectures the reduced osmotic modulus $(M_w/RT)(\partial\pi/\partial c)_T \equiv M_w/M_{app}$ is a characteristic function of the parameter $X = A_2 M_w c$, where A_2 is the second osmotic virial coefficient and c the concentration. This quantity X is proportional to c/c^*, where c^* is the concentration at which molecular coils start to overlap $(X = 1)$.

Approximate relationships for hard spheres [18] and linear flexible chains [19] have been derived which allow

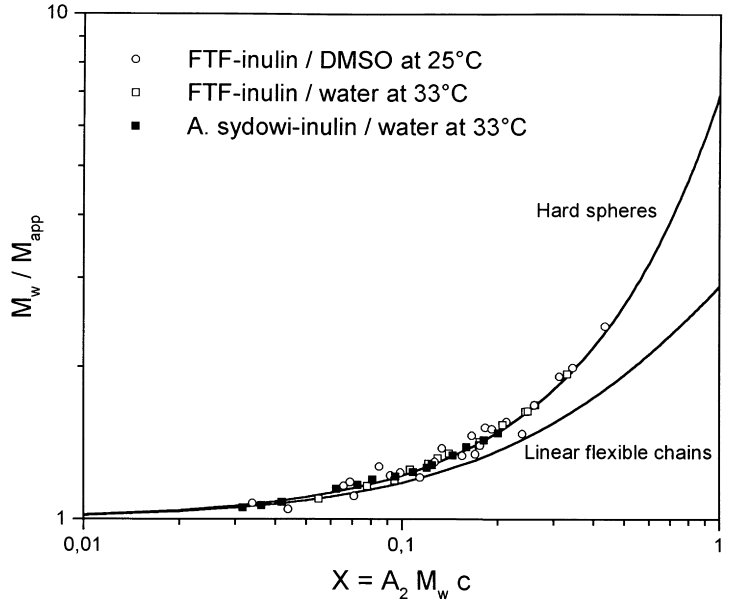

Fig. 4. Reduced osmotic modulus M_w/M_{app} versus X-parameter (full lines: theoretical curves, for detail see text).

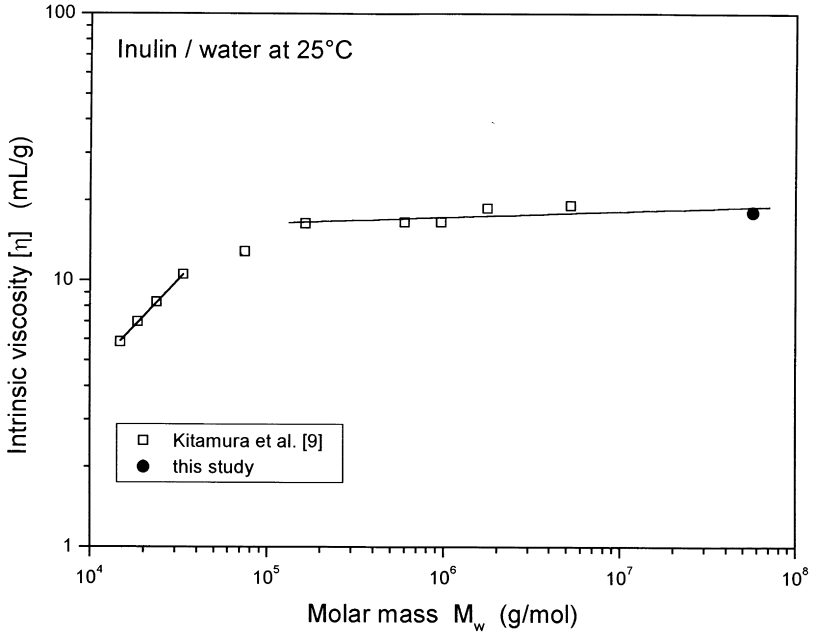

Fig. 5. Molar mass dependence of intrinsic viscosity for inulin, comparison of data from Ref. [9] and this study (lines: linear fit curves).

the description of M_w/M_{app} over the whole concentration range from dilute ($X \ll 1$) to semi-dilute solutions.

Fig. 4 shows the plot of the reduced osmotic modulus over the measured dilute ($X < 1$) concentration range in comparison with the theoretical curves for hard spheres and linear flexible chains. Although both curves do not differ very much in this concentration range, it is quite obvious that the experimental data lie on the curve for hard spheres. This suggests that both FTF-inulin and *A. sydowi*-inulin molecules assume a compact globular shape in both solvents.

The structure of macromolecules in dilute solution can also be characterized comparing the ratio ρ of root-mean-square radius of gyration (R_g) and hydrodynamic radius (R_h) to theoretical values. Whereas R_g depends on the mass distribution, R_h reflects the shape of the molecules. Therefore the dimensionless quantity $\rho = R_g/R_h$ is a very useful parameter to distinguish between compact structures like hard spheres (0.778), statistic coils of linear molecules (1.78) and rods (>2.0) [20]. R_h can be estimated from the translational diffusion coefficient D using the Stokes–Einstein equation $R_h = k_B T/(6\pi n_0 D)$, and D can be determined by dynamic light scattering technique [21].

From a preliminary measurement on one of our FTF-inulin samples in water at 25°C (SLS: $M_w = 54 \times 10^6$ g/mol, $R_g = 41$ nm) a hydrodynamic radius of $R_h = 54$ nm was determined.[1] The derived ρ value of 0.76 also indicates a globular shape of the macromolecules in dilute aqueous solution.

[1] Obtained with an autocorrelator equipped ALV/DLS goniometer, ALV-Laser Vertriebsgesellschaft m.b.H. (Langen, Germany).

3.2. Viscometry

From viscosity measurements the intrinsic viscosity $[\eta]$ of the inulin samples in dilute aqueous solution was evaluated. In the case of dilute polymer solutions, $[\eta]$ reveals the specific volume occupied by the individual molecule. Therefore from the dependence of intrinsic viscosity on weight average molar mass M_w the molecular shape can be discussed.

Expressed by Mark–Houwink equation

$$[\eta] = \text{const.} \times M_w^a \quad (1)$$

the value of exponent a is zero for spheres, below 0.5 for branched structures, in the range of 0.5–0.9 for random coils and 2.0 for rods.

The enzymatically synthesized inulin was available only in the range of molar mass from 30×10^6 to 90×10^6 g/mol. Therefore it was not possible to determine the $[\eta]$–M_w dependence only from synthesized inulin described here and in Ref. [6]. But the studies reported by Kitamura et al. [9] on inulin synthesized by *A. sydowi* conidia could be extended to a 10 times higher molar mass region.

Before starting the measurement with an Ubbelohde-type capillary viscometer the shear-rate dependence of the viscosity of the inulin solution has been investigated using a rotational viscometer. In the available range from 0 to 1000 l/s, no shear-rate dependence was observed, so that Newtonian behavior can be assumed. This was expected for globular particles and is therefore consistent with our results obtained by light scattering.

$[\eta]$ could be evaluated from both plots η_{sp}/c and $\ln \eta_r/c$ versus c, where η_{sp} and η_r are specific viscosity and relative viscosity, respectively. For $[\eta]$, a value of 18 ml/g was

Table 2
Results of determination of the linkage pattern (values in mol%)

Method	Methylation analysis		Reductive cleavage	
Sample	FTF-inulin	*A. sydowi*-inulin	FTF-inulin	*A. sydowi*-inulin
Terminal fructosyl units	4.4	5.3	6.1	5.4
2 → 1-linked fructosyl units	90.2	87.3	86.9	87.0
Under- and/or demethylated products[a]	–[b]	0.8	–[b]	1.2
2 → 1,6-linked fructosyl units	5.4	6.6	6.9	6.3

[a] Presumably from 2 → 1-fructosyl units.
[b] Not determined.

obtained which is in very good agreement with 16 ml/g for a fructan of 10^7 g/mol synthesized by *A. sydowi* conidia [11].

Fig. 5 shows the molar mass dependence of $[\eta]$ for inulin fractions reported by Kitamura expanded by the value for one of our high molar mass inulin samples. Two linear segments can be distinguished for which the exponent of the Mark–Houwink equation was obtained by linear regression of double-logarithmic plot $[\eta]$ versus M_w. For fractions having a molar mass below 50 000 g/mol, Kitamura et al. have determined:

$$[\eta] = 6.76 \times 10^{-3} \times M_w^{0.71}.$$

However, as the molar mass exceeded 10^5 g/mol, a very weak dependence of $[\eta]$ on M_w was found:

$$[\eta] = 12.6 \times M_w^{0.02}.$$

The existence of two distinct linear sectors of different slope in Fig. 5 suggests two structures. The exponent 0.71 points to a random coil behavior for samples with M_w below 50 000 g/mol. On the other hand, an exponent of 0.02 derived for molar masses higher than 0.16×10^6 g/mol is close to zero and therefore typical for a spherical particle.

In this connection, it is interesting to note the work of Stilava et al. [7], who examined the behavior in aqueous solution of fractions from branched *Streptococcus salivarius* levan. A plot of their data of M_w and $[\eta]$ yields two linear segments having exponents of 0.67 for $M_w < 10^5$ g/mol and 0.05 for $M_w > 10^5$ g/mol, respectively. These investigators reported that fractions of $M_w > 10^5$ g/mol behave as compact spheres, whereas for $M_w < 10^5$ g/mol the particles are best characterized as linear coils.

3.3. Determination of branching

From methylation analysis and reductive cleavage, it is evident that both FTF-inulin and *A. sydowi*-inulin are 2 → 1-linked fructans with about 6% of 2 → 1,6-branched residues. The relative ratio of about 5% of terminal fructosyl residues is in good agreement with a high molar mass branched structure for both polymers. The detailed results are shown in Table 2.

After methylation analysis, the corresponding partially methylated and acetylated mannitol and glucitol derivatives were identified by GLC and GLC–MS. In addition, small amounts of presumably undermethylated components were also detected. For reductive cleavage, slight demethylation cannot be excluded. Reductive cleavage gave the expected 2,5-anhydroglucitol and -mannitol derivatives. An additional product was detected, the mass spectrum of which indicates the structure of a 2,6-di-*O*-acetyl-1,5-anhydro-3,4-di-*O*-methyl-D-hexitol. This unexpected product is presumably formed from 2 → 1,6-branched fructosyl residues. The carboxonium ion formed after cleavage of the glycosidic bond can be intramolecularly attacked by the oxygen at C-6. By stereoselective rearrangement and the subsequent reduction, 1,5-anhydro-2,6-di-*O*-acetyl-3,4-di-*O*-methyl-D-mannitol is formed, in which the original C-1 of the fructose is now numbered C-6. This artefact was also detected and discussed by Spies et al. [22]. Due to its origin from 2 → 1,6-branched fructosyl residues, it was included in the quantitative evaluation. After acid hydrolysis up to 5% difructose-1,2′:2,1′-anhydrides were detected, which are formed when the sample is evaporated and therefore concentrated. These were also included in the quantitative calculation.

3.4. Discussion of polymer shape

Assuming a globular shape of the polymer in dilute solution according to Eq. (2) the diameter d of such a sphere can be estimated from M_w determined by SLS and $[\eta]$ obtained by viscosity measurements:

$$d_{visc.} = \sqrt[3]{\frac{6}{2.5}\frac{[\eta]M_w}{\pi N_L}} \quad (2)$$

On the other hand, using the value of R_g from SLS experiments according to Eq. (3) the sphere diameter can be calculated:

$$d_{SLS} = \sqrt{\tfrac{20}{3}} R_g \quad (3)$$

Applying Eqs. (2) and (3), for instance, to the FTF-inulin sample of $M_w = 57 \times 10^6$ g/mol, root-mean square radius of gyration in water of $R_g = 42$ nm (both determined by SLS) and intrinsic viscosity in water of $[\eta] = 18$ ml/g

(determined by viscosity measurements) reveal a sphere diameter of $d_{SLS} = 108$ nm and $d_{visc.} = 109$ nm, respectively.

The excellent agreement of these values determined with two different methods supports the assumption of spherical shape of synthesized inulin in dilute aqueous solution.

A compact globular conformation has usually been observed for highly branched levan [23] but not for linear molecules. Kitamura et al., who described *A. sydowi*-inulin to be essentially linear [9], related a compact conformation to strong intramolecular interactions between more distant residues of the same molecule. Our recent kinetic studies of FTF-inulin synthesis in batch indicated that the structure of the enzymatically synthesized inulin becomes more and more dense with increasing molar mass [6]. This seems to support the assumption of Kitamura and coworkers. But the amount of degradation products of terminal fructosyl units as well as fructosyl units linked via β-(2 → 1) and β-(2 → 6) position indicates that 5–7% monomer units of both FTF-inulin and *A. sydowi*-inulin constitute branch-points. This is in accordance with the value reported by Rosell and Birkhed [5] and contradicts the assumption of linear polymer chains [9–11]. From the determined amount of branches, it has to be concluded that branching occurs on average every 20 fructosyl units. Unfortunately, from the analysis of degradation products, it is not possible to distinguish between long and short chain branches. For a hyperbranched polymer (long chain branches), a globular shape in dilute solution would be expected. On the other hand, a high molar mass polymer with only one terminal fructosyl group every 20 repeat units (short chain branches) may be regarded as essentially linear. In that case, according to Kitamura et al., a globular conformation could be explained also with intramolecular interactions between distant residues of the same molecule.

The knowledge of the type of branching would help not only to discuss the origin of globular shape in dilute solution but also to understand the mechanisms of the inulin synthesis by the enzymes of *S. mutans* and *A. sydowi* conidia. To obtain detailed information about the type of branching, for instance, from the slope of the log–log plot of the R_g–M_w relationship, it would be necessary to compare the branched polymers with a linear sample of the same molar mass.

4. Conclusions

A globular conformation of high molar mass inulin synthesized by FTF of *S. mutans* and *A. sydowi* conidia, respectively, in dilute aqueous and DMSO solution was determined by HPSEC, light scattering and viscometry. The compact shape of these macromolecules is the reason for small root-mean-square radii of gyration considering the very high molar mass reported recently [6] and in this contribution.

Determination of 5–7% β-(2 → 6) branches reveals that these polysaccharides do not consist of linear chains as described for inulin synthesized by *A. sydowi* conidia [9]. The type as well as the origin of branching is not understood until now. A compact globular shape would be expected, for instance, for a hyperbranched or long chain branched polymer. But if only short chain branching like single terminal fructosyl units occur, the spherical shape of the molecules has to be explained by strong intramolecular interactions between more distant residues of the same molecule [9].

Comparing the R_g–M_w relationship for two inulin samples of similar molar mass but synthesized either by FTF from *S. mutans* or by *A. sydowi* conidia, it is shown for the first time, that these polyfructans with the same total amount of branches may have different branching architectures.

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