Structure Properties of Dextran. 3. Shrinking Factors of Individual Clusters

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ABSTRACT: Size exclusion chromatography in combination with multiangle laser light scattering and viscometry was applied for the study of the branched structures of commercial dextrans. Shrinking factors $g = R_{g,i}{}^2{}_{branch}/R_{g,i}{}^2{}_{linear}$ and $g' = [\eta]_{i,branch}/[\eta]_{i,linear}$ were calculated, where the radii of gyration of pullulan were taken as the reference for the derivation of g, For the calculation of the g' factor, a reference curve was constructed by linear extrapolation of the low molar mass dextran data toward higher molar masses. A power law behavior $g' = g^{b_\eta}$ was found with exponent $b_\eta = 0.71 \pm 0.05$ which is slightly larger than 0.60 as was estimated by Kurata et al. and found with endlinked polystyrene star molecules. The combination of both shrinking factors also allowed us to estimate the Flory draining parameter Φ as a function of molar mass and of g, respectively. The Φ factor was found to increase with the number of branching points of the macromolecules. A quantitative estimation of the number of branching points is possible for randomly branched materials by applying the Zimm−Stockmayer relationship. For a good fit the equation had to be modified by changing the asymptotic power from −0.5 to −0.72. The branching unit was found to have a molar mass of 29 000 g/mol, which corresponds for hyperbranched chains to a chain length of about 90 monomer units between two branching points. The effect of incomplete fractionation is discussed.

Introduction

In a previous paper,[1] we reported dilute solution properties from a number of dextrans prepared by different techniques. However, the characterization of the samples was not complete because we avoided the evaluation of the so-called shrinking factors g and g' from the nonfractionated samples and the individual fractions obtained by size exclusion chromatography (SEC). The reason for our procedure was that the evaluation of the SEC results required a rather expanded explanation. The shrinking factors are defined for the same molar masses of the branched and linear chains by the equations[2−4]

$$g_i = \frac{R_{g,i}{}^2{}_{branch}}{R_{g,i}{}^2{}_{linear}} \quad (1)$$

$$g_i' = \frac{[\eta]_{i,branch}}{[\eta]_{i,linear}} \quad (2)$$

and need reference curves for linear dextrans. In the above equations, the subscript i indicates dimension and intrinsic viscosity of the individual fractions, but the same relationships hold also for nonfractionated samples where the subscripts i have to be avoided. Dextrans are generally branched, and so far no linear dextran chains could be prepared by fermentation in a commercial scale. Some success was achieved by Schuerch[5] and Uryu[6] by cationic ring opening polymerization of 3,6-anhydride glucose, but the synthesis was laborious, and only fairly short chains could be prepared. Therefore, linear chains were not available in our study as a required reference. As an alternative the properties of linear pullulan could be taken.[7−9] Comparative studies with the low molar mass dextran revealed virtually the same molar weight dependence of the radius of gyration.[10] A few other characterizations of dextrans were made earlier with limited instrumental possibilities.[11−14] A detailed analysis, similar to the present study, was made previously by Kuge et al.[11] They applied common gel permeation chromatography and determined first the product $M_{wi}[\eta]_i$ from each slice on the basis of a universal calibration function $M[\eta]$ as a function of the elution volume v_e, which was established for a number of linear and branched glucans in water. The molar mass M_{wi} of the fractions in each slice was then determined with the aid of the molar mass dependence of the intrinsic viscosity, that was established with nonfractionated and fractionated dextrans. Within experimental errors, no differences between fractionated and nonfractionated samples were found. As will be shown below, their results very satisfactorily agree with those published by Senti et al.[13] However, so far known to us, the present work and that of Johann[10] are the first attempts where molar mass, radius of gyration, and intrinsic viscosity were simultaneously determined by online combination of detectors for the refractive index increment (RI), multiangle laser light scattering (MALLS), and viscosity (VISC) applied to the water soluble dextran. No calibration function was needed, and all quantities could be determined without any assumption. Quite an interesting characterization was made also by Gekko and Noguchi.[15] Among other properties they also tried to establish the molar mass dependence of the intrinsic viscosity. Unfortunately, they determined the number average molar mass but found a significantly larger exponent than those found by the authors in refs 11−13. Such behavior has been

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known for a long time, because despite careful fractionation it is virtually impossible to prepare sufficiently narrow distributions with branched materials. For this reason, their data could not be used in this study.

Because of the low degree of branching in dextran the lowest fractions of a low molar mass dextran should exhibit linear chain behavior. For this reason we took as reference curve pullulan from the measurements Kato et al.,[7] Buliga and Brant.[8] and Nordmeier,[9] who obtained a remarkable agreement in their results. There is no reason to expect identical dimensions for pullulan and linear dextran, because the former contains a ratio of two α(1,4) bonds to one α(1,6) bond whereas in dextran there are only α(1,3) bonds in the chain. However, Kato et al. and Buliga and Brant proved typical good solvent behavior for pullulan. The same *behavior* can safely be assumed also for (linear) dextrans but different absolute values for the two polysaccharides are to be expected. Therefore, the radii of gyration of the linear pullulan samples were multiplied by a factor of 1.22 to bring the pullulan data to the dimensions of short chain dextrans. This factor corresponds to the polydispersity of a Schulz−Flory distribution, which we thought should be introduced, because the pullulans were narrowly distributed, but the same could not be expected for the dextrans even after fractionation by SEC. However, our procedure is merely an empirical approach.

The situation was more complex with the intrinsic viscosities because no calibration curves for linear dextran fractions were available. In this case, we relied on the lowest molar masses in the relationship of the intrinsic viscosity as a function of the molar mass and assumed these as being linear. This curve was shown in Figure 4 of part 2[1] and is reproduced in this paper in Figure 3. The exponent in this power law behavior of the intrinsic viscosity is $a_\eta = 0.62$, that is characteristic for linear chains in a marginally good solvent. Applying scaling laws[16] one finds with this exponent a_η a value of $\nu = 0.54$ in the molar mass dependence of the radius of gyration, that satisfactorily agrees with the value of $\nu = 0.57$ measured by Nordmeier,[9] $\nu = 0.58$ by Kuge et al.[7] and $\nu = 0.589$ by Buliga and Brant[8] for pullulan.

The main purpose of the present paper is related to two aspects: (1) the applicability of the Zimm−Stockmayer equation for the shrinking factors g, and (2) the relationship between g' and g.

(1) So far the shrinking factors as derived by Zimm and Stockmayer[3] refer to molecularly uniform fractions from *randomly branched* macromolecules. Dextran, however, is much more densely branched and belongs to the class of so-called *hyperbranched* macromolecules of the general type $A<\frac{B_2}{B_1}$. Because of the stringent constraint that group A can react only with one of the two B groups, a very different size distribution of the molar mass and a considerably lower polydispersity are obtained. ($M_w/M_n \propto M_w^{1/2}$, hyperbranched; $M_w/M_n \propto M_w$, randomly branched). It is likely that also the isomer distribution (different branching densities for the same molar mass) will differ. Therefore, we wished to check whether the equation for g_i of randomly branched chains also holds for this type of polymer.

(2) Clearly there should be a correlation between g' and g. For star-branched polymers Zimm and Kilb[17] found the simple relationship $g' = g^{1/2}$, but such behavior was never observed experimentally.[2] Kurata et al.[18] expanded this relationship to a power law $g' = g^{b_\eta}$ and estimated the exponent from literature data of star and comblike molecules to $b = 0.60$, a value that indeed was approximately found for fractions of randomly branched samples.[19,20]

In this paper, we evaluated the two shrinking factors as a function of molar mass and checked the dependence between g' and g. Furthermore the Zimm−Stockmayer[3] prediction for the g factor as a function of molar mass was examined. As is known for long, the intrinsic viscosity is related to the radius of gyration and the molar mass via the Fox−Flory relationship[21] which for monodisperse fraction is given as

$$[\eta]_i = \Phi_i \frac{R_{g,i}^3}{M_i} \tag{3}$$

The parameter Φ_i depends on the hydrodynamic interactions and increases with the segment density due to branching. Therefore, one has to distinguish between $\Phi_{i,\mathrm{branch}}$ and $\Phi_{i,\mathrm{linear}}$. In the last part of our paper, we checked whether and how $\Phi_{i,\mathrm{branch}}$ varies with molar mass.

Experimental Section

The samples were the same as given in ref 1. An extensive description of the origin and preparation of the used samples and all details about the size exclusion chromatography were given there. Also the data from size exclusion chromatography (SEC) were the same and were used here without modification. Altogether 14 samples have been thoroughly characterized, but only five samples from Sigma (D1, D2, D3, D4, D5) were selected for this study for two reasons. For the two other Sigma samples, D6 and D7, the radii of gyration were too low and below the confidence range for reliable radii determination by light scattering. The other samples, labeled in ref 1 as Dd1−Dd5 are fractions obtained by a controlled acid degradation of D1 in aqueous methanol suspensions. (Details of the preparation are given in ref 1.) These were also evaluated and gave a good agreement of the viscosity data, but not for the radii. We later found out that the cleavage rate of α(1,6) bonds differs from that of the α(1,3) bonds and led to a change in the branching density. To avoid confusion, these data are therefore not shown.

Table 1 gives a list of the weight average molar masses and the radii of gyration for the nonfractionated samples D1−D5, determined by two instruments of static light scattering and by SEC in combination with the multiangle laser light scattering (MALLS) detector and an on-line viscometer (VISC).

Three columns in a series were used, two Aquagel-OH Mixed, 8μm columns from Polymer Laboratories, Heerlen, Netherlands, and one Suprema 10000, 10μm column from Polymer Standards Service in Mainz, Germany. For the two highest molar mass dextrans, a Suprema 30000, 20μm column was used in addition. The injection volume was 50 μL, and the flow rate was 0.5 mL/min. Further details are given in ref 1.

Results

As already mentioned, in the present paper we wish to examine the behavior of shrinking factors for nonrandomly branched (i.e. hyperbranched) macromolecules. Furthermore, we were interested in the dependence on the branching density, their molar mass dependence and the influence of branching on the hydrodynamic interaction. Table 1 gives a list of the essential molar parameters of the samples used.

Shrinking Factors *g* for the Radius of Gyration. Figure 1 shows the result from five fractionated Sigma

Table 1. Some Molecular Parameters of Commercial Dextrans (Sigma D1−D7) and Degraded Dextrans (Dd1−Dd5)[a]

sample	$10^{-4}M_w$ (g/mol)	10^5A_2 (mol ml/g^2)	$R_{g,LS}$ (nm)	R_h (nm)	[η (mL/g]	$10^{-4}M_{w,SEC}$ (g/mol)	$R_{g,z}$ (nm)	$10^{-4}M_n$ (g/mol)
D1	268.	5.4	47	47	61.0	278.	44	8.43
D2	49.4	17.6	21	17	53.1	44.9	25	17.0
D3	34.4	20.2	19	15	46.1	27.8	22	3.94
D4	14.9	32.8	12	11	35.0	14.2	19	4.87
D5	6.37	45.6		10	26.3	6.53	11	4.2
D6	4.20	42.2		5	20.9	3.31	10	2.09
D7	1.04	74.9		3	8.6	0.83		0.48
Df1	295.	5.0	45	38	67.8	334.	49	18.2
Dd1	147.	9.3	33	29	62.1	129.	33	19.2
Dd2	56.5	15.1	29	26	49.8	58.3	30	12.2
Dd3	16.1	21.9	14	11	28.9	15.3	23	3.71
Dd4	8.30	38.0		9	18.1	4.3		1.59
Dd5	7.20	37.6		9	16.4			2.24

[a] The sample labeled Df1 is a middle fraction form D1 that was obtained by fractionated precipitation from an aqueous solution with methanol. The data are the averages from measurements which were made with an SOFICA light scattering instruments, equipped with a HeNe laser ($\lambda_0 = 632{,}8$ nm). The hydrodynamic radii were determined from diffusion coefficients using the Stokes−Einstein relationship. The required dynamic light scattering measurements were made with an ALV photogoniometer equipped with an ALV 5000 autocorrelator. The number average molar mass was determined by end group determination of the reducing end via the Nelson−Smogyi method. The intrinsic viscosities were measured with an automatic viscometer, using a capillary of 0.68 mm in diameter. For further details, see ref 1.

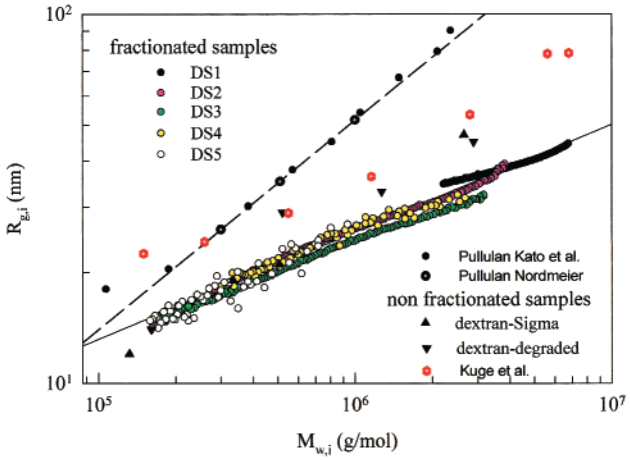

Figure 1. Molar mass dependence of the radius of gyration obtained with the five Sigma samples DS1, DS2, DS3, DS4, and DS5, using the SEC−MALLS technique. The data form one common line with a slope of 0.3, corresponding to an apparent fractal dimension of $d_{f,app} = 3.33$. The dashed line represents the molar mass dependence of pullulan in water,[7−9] adapted to the data of dextran at low molar mass. The filled circles and those with a white dot are due to Kato et al.[7] and Nordmeier,[9] respectively. The filled triangles up and down represent the *z*-average of the radius of gyration of the nonfractionated Sigma and degraded samples, respectively;[1] the hexagonal symbols with a white dot represent data by Kuge et al.[11]

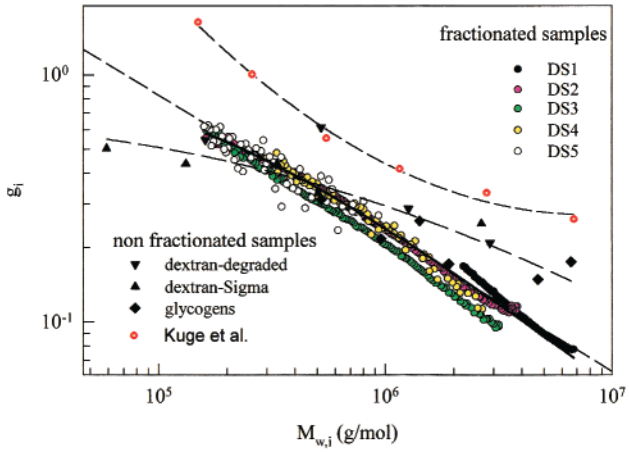

Figure 2. Molar mass dependence of the *g* factor resulting from Figure 1 for the five Sigma dextrans. The light dashed line is the forced linear fit through all the points, with a slope −0.56. The heavy line represents the fit with eq 8, with an exponent of −0.72. Data for the nonfractionated dextrans (black triangle up and down) and for nonfractionated glycogens[21] (black diamonds) are also shown together with the results from Kuge et al.[15] Notably the data of the nonfractionated branched glucans form one common curve. The heavy dashed line was drawn to guide the eye.

dextrans, and for comparison, also the data for the nonfractionated samples are shown. The dashed line is the curve for linear pullulans[7−9] reduced by a factor as mentioned in the Introduction. The corresponding *g* factors are shown in Figure 2 as a function of the molar mass. Within experimental errors the curves for the five samples coincide and may be approximated by a power law with exponent −0.56. A somewhat different behavior is obtained for the nonfractionated samples which is indicated by the long dashed line. Interestingly the data for nonfractionated glycogens[22] lie on the same curve for the fractionated dextrans than the nonfractionated dextrans.

Shrinking Factors *g′* for the Intrinsic Viscosity. The increase of intrinsic viscosity with molar mass for the five indicated samples is presented in Figure 3. With the exception of the lowest fractionated curve the results from the other samples coincide up to a molar mass of about 10^6 g/mol. At larger molar masses, splitting occurs which might indicate differences in branching density. Interestingly the data of the nonfractionated samples agree very well with the fractionated curves. This also holds for the fractions published previously by Kuge et al.[11] and by Senti et al.[13] For the samples prepared in our laboratory by special acid degradation of sample D1 (see ref 1) also a fairly good agreement is obtained but with a larger scatter, arising from the fact of different cleavage rates of α(1,6) and α(1,3) bonds. The data are not shown.

Figure 4 presents the *g′* values derived from the curves given in Figure 3. There is considerable scatter in the low molar mass region, but on the whole, a reasonable dependence is observed. At the moment we are not clear what the origin is of this scatter, which seems to be rather systematic.

Interrelation between *g′* and *g*. The *g′* factor is often easier to measure. It also depends on the number

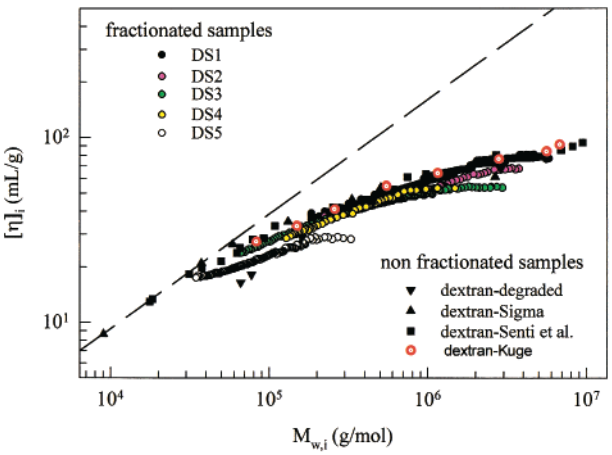

Figure 3. Intrinsic viscosity dependence on the molar mass for the same samples as shown in Figure 1. The results were obtained from SEC−MALLS in combination with a viscometer (VISC.) The filled triangles up and down correspond to the nonfractionated samples and the filled circles with a dot and squares are data from Kuge et al.[11] and Senti et al.,[13] respectively. The dashed line represents the initial slope at small molar masses, which may be related to linear dextrans.

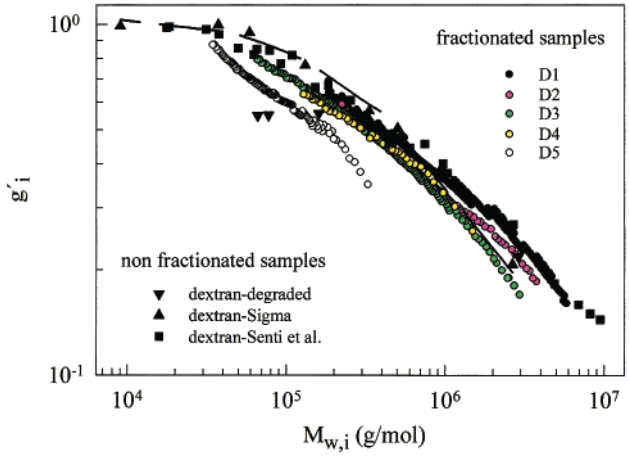

Figure 4. Molar mass dependence of the g'-factor resulting from Figure 3 for the five fractionated Sigma dextrans. For comparison, data for nonfractionated dextrans are also presented (Sigma dextran, black triangle up; degraded dextrans, black triangle down; Senti et al.[13] data, black squares). The heavy dashed line is a fit curve through the nonfractionated samples.

of branching points per macromolecule, but the dependence is more involved than for the g factor because the intrinsic viscosity is in addition affected by hydrodynamic interactions. For this reason it is of interest to establish a relationship that correlates g' to g. For star-branched macromolecules Zimm and Kilb[16] found in a theoretical study $g' = g^{1/2}$, but at present there is no theory for randomly and hyperbranched macromolecules. Figure 5 shows the experimentally determined relationship. Despite of considerable deviations this empirical relationship can be expressed by a power law as

$$g' = g^{b_\eta} \tag{4}$$

with an exponent $b_\eta = 0.71 \pm 0.05$. Satisfactorily, the relationship starts at the coordinates (1,1), which means no evidence for branching at low molar mass in both, the static and viscosity properties.

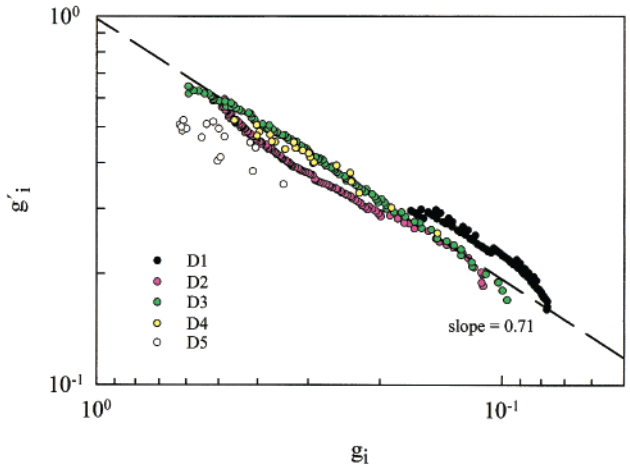

Figure 5. Viscosity contraction factor g' as a function of the geometric contraction factor g for the fractionated dextrans. According to eq 4, a common line with a slope of 0.71 ± 0.05 is found. The experimental data fulfill the condition $g = g' = 1$ at low molar mass and give no indication for branching in that molar mass region.

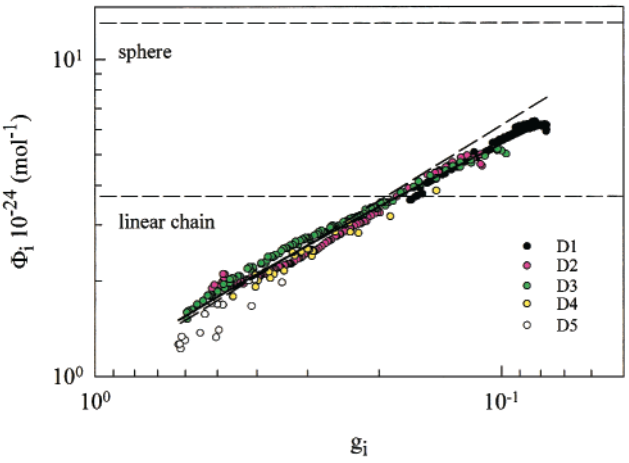

Figure 6. Dependence of the draining Φ factor on g for the fractionated Sigma dextrans. The curve is a fit through all the points and the dashed line corresponds to eq 6. The values for linear chains ($\Phi_{coil} = 3.69 \times 10^{24}$ mol^{-1}) and for spheres ($\Phi_{sphere} = 13 \times 10^{24}$ mol^{-1}) are also shown as parallel dashed lines. The slopes of 0.78 and 0.70 correspond to linear fits of the initial and the terminal parts, respectively, and may indicate a weak curvature (but note the experimental error).

Flory Factor Φ_{branch}. Having measured the viscosity and the radius of gyration (or g' and g), one obtains with eq 3 and the exponent b_η from Figure 5

$$g'(M_i) = g(M_i)^{0.71} = \frac{\Phi_{i,branch}}{\Phi_{i,linear}} g(M_i)^{3/2} \tag{5}$$

or

$$\Phi_{i,branch} = \Phi_{i,linear} g(M_i)^{-0.79} \tag{6}$$

Figure 6 shows the dependence of Φ factor on g for the fractionated Sigma dextrans. The dashed line through the points corresponds to eq 6.

The molar mass dependence of the Φ parameter is given in Figure 7 for the SEC fractions and for nonfractionated dextrans. In addition the data from degraded, nonfractionated starches[23] and glycogen[22] are given.

Discussion

Five samples of dextran were analyzed by the combined SEC−MALLS−VISC technique. These curves show some scatter, but on the whole one can say, at least for the radii of gyration $R_{g,i}$ and the g factor, common curves are obtained. For the viscosity a small systematic shift with M_w may be recognized, but even there the deviations are within, probably systematic, experimental error. The g and g' factors evidently approach unity at a molar mass of $M_{w,i} \cong 10^4$ g/mol, which is the value for a sample with no branching points. These findings indicate that chains shorter than about 60 monomer units in length are not significantly branched.

***g* Factors.** A more precise estimation of the number of monomer units between two branching points can be made on the basis of the Zimm−Stockmayer[3] equation for the g factor, which in terms of branching points is given by

$$g_i = \left[\left(1 + \frac{n_i}{7}\right)^{1/2} + \frac{4n_i}{9\pi}\right]^{-1/2} \quad (7)$$

where trifunctionality of the monomer was assumed. n_i denotes the number of branching points per molecule. This equation can be converted in terms of molar mass as follows

$$g_i = \left[\left(1 + \frac{M_i}{7M_0}\right)^{1/2} + \frac{4M_i}{9\pi M_0}\right]^{-1/2} \quad (8)$$

where M_0 is the molar mass of one branching unit, i.e., the branching point plus the attached chains between the branching points. For details see below.

For large molar masses eqs 7 and 8 approach a power law dependence with an exponent of −0.5. However, a much steeper decrease of g' with $M_{w,1}$ is found. A good fit was obtained if the exponent of −0.5 was changed to a free fit parameter. The best fit was obtained with an exponent of −0.72, resulting in $M_0 = 29\,000$ g/mol (solid line in Figure 2). A reasonable agreement is obtained. This molar mass corresponds to 179 glucose units per branching unit (the molar mass of the anhydroglucose unit AGU is $M_{AGU} = 162$ g/mol). For an estimation of the chain length between two branching points, two models have to be distinguished. For the randomly branched material, one has $l_{b,random} = (2/3)DP_0 = 119$, while for the hyperbranched model one has $l_{b,hyper} = (1/2)DP_0 = 89$ (see Figure 8). The obtained values for l_b can only be estimations, because of the empirical change of the original Zimm−Stockmayer equation. It is not clear to which extent this maneuver changes the value of M_0. We have to emphasize that the change of the exponent is solely empirical, and the physical basis is not known to us. A similar treatment was applied to the results from glycogen, but there an exponent of −0.3 was required.[22] One possible reason for the required change of the Zimm−Stockmayer formula may be seen in the hyperbranched structure. Very likely the distribution of branched isomers (i.e., branched clusters of the same molar mass, but different branching density) is more narrow for the hyperbranched than for the randomly branched materials. Unfortunately, we have not been able to apply the Zimm−Stockmayer technique to the calculation of individual hyperbranched clusters as was used to derive eq 7. The molar mass distribution of hyperbranched materials is much more complex, as was published recently.[24]

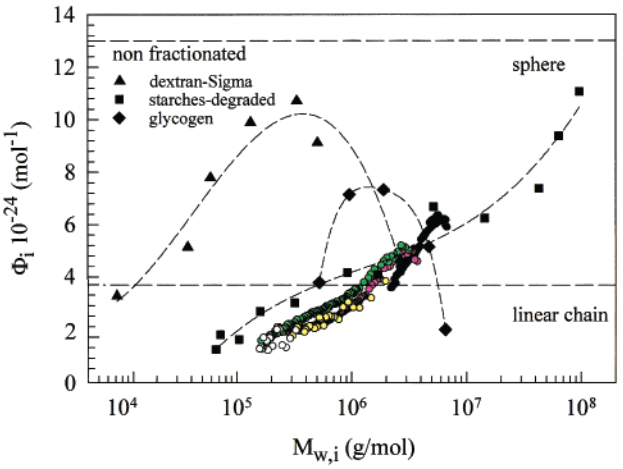

Figure 7. Molar mass dependence of the Φ parameter for the SEC fractions (the symbols are the same as in the other Figures) and for nonfractionated dextrans (triangle up). In addition the data from degraded, nonfractionated starches[22] (black squares) and glycogen[21] (black diamonds) are shown.

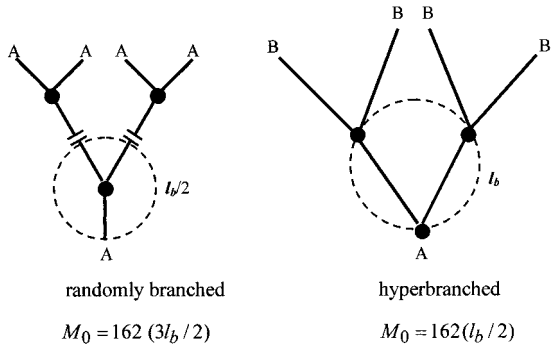

Figure 8. Repeating units (enclosed by dashed circles) in randomly branched and hyperbranched macromolecules, respectively. l_b denotes the chain length between two branching points expressed in number of anhydroglucose units. The sign = indicates the coupling positions between two A_3 units. The relation between the molar mass of the branching unit M_0 and the length of the chains connecting two branching points is given by the expression underneath the drawings. The molar mass of the anhydroglucose unit is $M_{AGU} = 162$ g/mol.

A completely different behavior was obtained for the nonfractionated dextrans, but surprisingly a good agreement was found with the data of nonfractionated glycogens. No explanation can be offered.

As already mentioned, the whole set of data could also be fitted by a simple power law over the whole region, but now with a slope of −0.56 instead of −0.50, required in eq 8. This force fit results in a systematic deviation at low M_{wi} to too high a molar mass M_0. (Note, this overall exponent must differ from the exponent in eq 7, as it neglects the influence of the low molar mass dependence). The chain length between two branching points of about 90 anhydroglucose units would correspond to a branching density of only 1.1%, and has to be considered as effective long-chain branching. Thus the remaining 3% branching has to be assigned to short-chain branching.

***g′* as a Function of *g*.** Not all laboratories have the full set up for measuring $R_{g,i}$ and $[\eta]_i$ as a function of the molar mass. Often only a low-angle light-scattering (LALLS) detector in combination with a viscosity detector are available. The g' factor should contain the same information of the branching density, but it also depends on the draining factor Φ_i. To convert the g' factor into

the g factor, a simple power law is mostly assumed, as given by eq 4. The empirically determined slope of Figure 5 is with $b_\eta = 0.71 \pm 0.05$ larger than the estimated one of $b_\eta = 0.60$ by Kurata.[18] For endlinked three arm polystyrene star molecules a value of $b_\eta = 0.663$ was obtained.[19] Similar results were recently obtained by Degroot et al. with branched polyethylene.[20] The somewhat larger exponent in our experiment may be a result of hyperbranching, but on the whole, the Kurata estimation is not too bad.

In this context, earlier estimates of the branching density by Kuge et al.[11] are of interest. These authors carried out SEC. The weight average molar masses were determined from a generalized calibration curve and the intrinsic viscosity from the Kuhn−Mark−Houwink calibration curves established with fraction from some water soluble polymers. They evaluated the intrinsic viscosity curve on the basis of the Zimm−Stockmayer eq 7 in combination with the estimated Kurata exponent of $b_\eta = 0.60$ for the relation (eq 4) between g and g'. The downward curvature of the experimentally determined intrinsic viscosity was found by this analysis being caused by an *increase* in the long chain branching as the molar mass is increased. However, this conclusion is based on the two assumptions that the Zimm−Stockmayer relationship remains valid, also for the hyperbranched fractions and the estimated Kurata exponent $b_\eta = 0.6$. In our study we tried to avoid assumptions as much as possible. The larger negative exponent of −72 in the molar mass dependence of the g factor may well be an effect of increasing long-chain branching content, but likewise could be the result of limited applicability of eq 7.

Φ as a Function of *g*. Both the Φ and the g factors are evidently functions of the number of branching points. Thus, a rather defined correlation of this draining factor with the shrinking parameter can be expected. This indeed is observed as shown in Figure 6. The data do not exactly follow a power law, as the curve flattens slightly before the limiting value of a hard sphere might be obtained, but this limit still remained a long distance away from the experimental points. This means that the branching density, even of the largest samples, is fairly low and remains in the intermediate region of the segment density distribution of a flexible linear chain and the uniform density distribution in a hard sphere.

Change of Φ_i with Branching. As already mentioned, the Φ_i factor describes the depth of solvent penetration (draining) into the clusters. This draining should be strongly reduced with increasing segment density as a result of branching and finally should approach the limiting value of a sphere with an impenetrable surface. The expected increase is indeed observed as the particles grow in mass, but the hard sphere behavior is not yet reached. The dashed line in Figure 7, denoted as linear chain, represents the asymptotic behavior of very large linear chains. For molar masses smaller than $M_i = 10^4$ g/mol also for linear chains a larger draining in agreement with theory is observed. It may be denoted that the data for degraded starches follow the same curve, when a polydispersity correction is applied, which is given[23] by eq 9, where ν

$$\Phi = \left(\frac{M_z}{M_w}\right)^{3\nu}\left(\frac{M_w}{M_n}\right)^{3\nu-1}\langle\Phi\rangle \qquad (9)$$

is the exponent from the molar mass dependence of the radius of gyration and $\langle\Phi\rangle$ denotes the measured average value. Surprisingly, a completely different behavior is observed with the nonfractionated dextrans. We mention this observation without offering an explanation.

Remarks on Degraded Dextrans. We also evaluated the SEC diagrams of the samples Dd1, Dd2, and Dd3, which were obtained by a special acid degradation of the sample D1 (see ref 1). The sample denoted DF1 represents a middle fraction obtained from D1 that was obtained by stepwise precipitation on adding methanol to the aqueous solution (for details, see ref 1). Unfortunately, the size exclusion fractionation of these samples was done only with a two-column set. Later we discovered[1] that this combination was not sufficient for a satisfactorily fractionation. For the values of the intrinsic viscosity we observed very good agreement, but no good results were obtained for the radius of gyration and the corresponding shrinking factor. These data demonstrate the effect of incomplete fractionation, but the poor agreement with the nondegraded samples is also caused by the not fully random degradation of the samples as was pointed out already at the beginning of this paper.

Conclusion

The key parameter for a determination of number of branching units is the shrinking parameter of dimensions, g. A number of laboratories still have only a viscosity detector in combination with the SEC device, and other in addition a low angle laser light scattering (LALLS) detector. In the first case the correct molar mass can be determined via a generalized calibration function of $[\eta]M$ vs the elution volume. Together with the measurement of the intrinsic viscosity, the molar mass and the Kuhn−Mark−Houwink value can be obtained. This was the procedure used by Kuge et al.[11] In the second case the molar mass and the intrinsic viscosity are determined directly online with the RI detector, but still only the g' shrinking factor for the viscosity can be derived with the appropriate reference curve for the linear chains. This technique was used, for instance, by Weissmüller and Burchard.[19] To obtain the g parameter, an empirical relationship has to be assumed. It is only recent that essential molecular parameters can be measured directly online with SEC. In combination with the MALLS detector also the radius of gyration and consequently also the g parameter can be measured directly without assumptions.

It was of special interest whether a relation between g' and g could be found, that remains universally valid for all types of branched polymers. From earlier measurements with uniform star-branched macromolecules, the existence of universality clearly had to be denied.[2] A difference in the exponent b_η also exists between randomly branched and hyperbranched macromolecules, but the change to higher exponents, as observed with the star molecules,[2] is only weak (from 0.6 to 0.7) when passing from randomly branched to the hyperbranched materials. However, a clear difference was observed for the molar mass dependence of the g factors and makes all determinations for the number of branches per molecule on the basis of the Zimm−Stockmayer relationship questionable. Conclusions drawn by Kuge et al.[11] on an increase of long-chain branching density with increasing molar mass are therefore not really convincing. In principle, the combination of SEC with

MALLS and viscosity measurement allows us to carry out an exhaustive determination of the structure of macromolecules in solution. A serious limit, however, is given by the fairly poor resolution power of the gel columns, in particular for the water soluble polymers, as was demonstrated in ref 1. More reliable results may be obtainable soon from field flow fractionation techniques in combination with the MALLS and viscosity detectors. Corresponding experiments are in progress.

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