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Solution properties of β-D-(1,3)(1,4)-glucan isolated from beer

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β-D-(1,3)(1,4)-glucan isolated from beer, and in comparison to this, a purchased one isolated from barley, were investigated by combined static and dynamic light scattering and rheological methods. The weight average molecular weight of β-D-(1,3)(1,4)-glucan of beer and barley was determined as 175,000 ± 5000 Da. The particle weight of β-D-(1,3)(1,4)-glucan strongly depends on concentration, solvent, temperature and external forces. The aggregates are assumed to be caused by association via hydrogen bonds. A specific effect resulted when maltose was included at different concentrations. Near 5% (w/v), a minimum of aggregation was found. The findings from static and dynamic light scattering and from viscometry indicate fringed micelle formation, a model which is described in detail. Variation of pH between 2 and 11 did not influence the viscosity.

INTRODUCTION

β-D-(1,3)(1,4)-glucan is a linear glucosidic polysaccharide with (1,4)-linkage sequences, interrupted by single (1,3)-linkages (Wood *et al.*, 1991). Hydrolysates obtained by treatment of lichenase (endo-β-D-(1,3)(1,4)-glucanase (EC 3.2.1.73)) consist of approximately 60% 3-*O*-β-D-cellobiosyl-D-glucose, 30% 3-*O*-β-D-cellotriosyl-D-glucose and 10% higher 3-*O*-β-D-cellooligosyl-D-glucose (Erdal & Gjertsen, 1967; Woodward *et al.*, 1983*b*; Wood *et al.*, 1994). The ratio of (1,4)/(1,3) linkages is 2,3–2,7. Figure 1 shows a section of the β-D-(1,3)(1,4)-glucan chain. Due to the similarity of β-D-(1,3)(1,4)-glucan with cellulose, quasi-crystalline regions of associated (1-4)-blocks are formed. Single (1,3) linkages prevent crystallization of longer chain sections and cause apparent water solubility of the β-D-(1,3)(1,4)-glucan. Because of this, β-D-(1,3)(1,4)-glucans tend to form gels because of partial association via hydrogen bonds. This property results in an important function of β-D-(1,3)(1,4)-glucan in plant cell walls. It facilitates the cell wall flexibility during growth and germination (Franz, 1991). β-D-(1,3)(1,4)-glucan is an important constituent of barley endosperm cell walls (Fincher, 1975; McCleary & Nurthen, 1986; Palmer, 1992). There it occurs free, or carboxylate bonded to α-aminoacids (Forrest & Wainwright, 1977*a*; Bamforth, 1981). During malting and mashing β-D-(1,3)(1,4)-glucan is enzymatically degraded and dissolved (Scott, 1972; Bamforth, 1981). The water soluble fraction is released in beer during brewing. Depending on technical processing, formation of a β-D-(1,3)(1,4)-glucan gel may occur during fermentation and storage (Bamforth, 1982). The gel causes enormous difficulties in beer processing (Gjertsen, 1970; Letters, 1977; Wagner *et al.*, 1988), blocking filter membranes and harming the economy of the process.

A wide range of different molecular weight, water soluble β-D-(1,3)(1,4)-glucans is reported in the literature. It extends from 2000 up to 40,000,000 Da (Igarashi & Sakurai, 1965; Erdal & Gjertsen, 1967; Igarashi & Amaha, 1969; Bathgate, 1974; Forrest & Wainwright, 1977*b*; Woodward *et al.*, 1983*a*; Foldager & Jørgensen, 1984; Wackerbauer & Anger, 1988; Strobl, 1991).

Similar studies have been published previously on β-D-(1,3)(1,4)-glucan from oat aleurone by Vårum *et al.* (1991, 1992). However, it should be emphasized that this β-glucan deviates significantly in its primary structure (Dais & Perlin, 1982; Åman & Graham, 1987; Wood *et al.*, 1991, 1994) and the resulting association behaviour from the polysaccharide studied here. For example, Vårum *et al.* (1991, 1992) reported a pronounced polyelectrolyte character, which is not found with the β-D-(1,3)(1,4)-glucan from barley endosperm. In fact, the same behaviour is not expected, since the polymer in the aleurone must have a different biological function compared with the β-D-(1,3)(1,4)-glucan which will be discussed in this paper.

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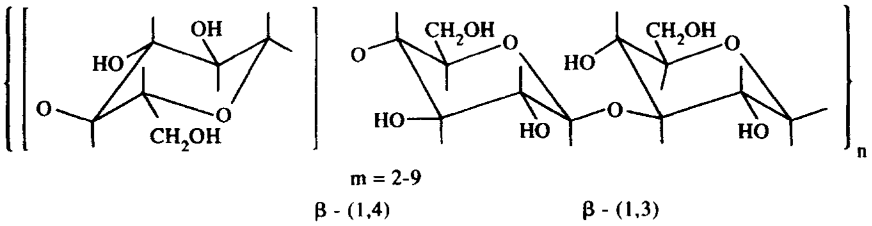

Fig. 1. Chemical structure of β-(1, 4)(1, 3) glucan. The average sequence length of the β-(1,4) sections is 2.3–2.7 which are connected via single β-(1, 3) glycosidic bonds.

In the present work, physico-chemical methods are applied to investigate the solution properties and molecular shape of a β-D-(1, 3)(1, 4)-glucan (simply named β-glucan) sample isolated from beer, which is able to form gels in beer and known to arise from barley endosperm cell walls (Igarishi & Sakurai, 1965; Igarashi & Amaha, 1969).

MATERIALS AND METHODS

Material

Isolation

β-glucan was isolated from filtered beer by cross-flow filtration using a membrane 0.2 μm pore size, where a 7-fold enrichment was obtained. Gel formation was induced by pretreatment of the enriched beer with a laboratory homogenizer according to a method described by Letters (1977). After storage for 7 days at −4°C, a gel was formed, which could be separated by centrifugation at 3400*g*. After washing 10 times with water and repeating centrifugation, a pure substance was obtained, which after freeze-drying contained less than 0.05% nitrogen. For comparison, a purchased β-glucan isolated from barley (Sigma®) was investigated.

Preparation of solutions

Aqueous solutions were prepared from highly purified water (Filtration-system, Millipore-Corp.). For homogenization, the samples were heated and stirred for 20 min at 80°C. Next, they were filtered through a 0.45 μm pore size membrane filter (Millipore®). The β-glucan was dissolved in 90% dimethylsulfoxide (DMSO), 2 M guanidinium hydrochloride (GHCl) and urea solutions, because of the ability of these substances to break hydrogen bonds. Furthermore, solutions of maltose at different concentrations were used as solvents to simulate a substance which usually occurs in wort. Unless stated otherwise, the concentration of β-glucan in solution was 0.1% (w/v).

An additional method for influencing interactions of associating polysaccharide molecules is obtained by dissolving the polysaccharide in metal complexing solvents. Cuoxam (copper(II) tetrammine-hydroxide) and cadoxen (cadmium ethylenediamine) are commonly applied to cellulose (Staudinger, 1932; Gralen, 1944; Henley, 1969). The cuoxam solvent was prepared by dissolving 5 g $Cu(OH)_2$ and 1.5 g CuCl in 25% NH_3 solution at a temperature of 5°C. The solvent was handled under Ar- or N_2-atmosphere to avoid oxidation.

Cadoxen was synthesised as described by Henley (1969). 50 g CdO were dissolved in 28% aqueous ethylenediamine solution. After centrifugation the solution was diluted with 0.35 M NaOH to 5% Cd.

To separate insoluble particles from the solutions, the floating-technique was applied i.e. the scattering cell containing the solution was set in a centrifuge beaker that was filled with an aqueous CsCl solution of a concentration such that the cell started to float (Dandliker & Kraut, 1956). The samples were centrifuged at 12,000 r.p.m. for 30 min in a Beckman ultracentrifuge L5-50B using a swinging rotor.

Derivatization by carbanilization

To avoid aggregation and network formation, β-glucan was transformed into the β-glucan tricarbanilate derivative with phenylisocyanate as described by Aberle (1993). Total derivitization was realized by repeating the reaction until the correct nitrogen content (8.13% N_2) was obtained. In the first step we obtained only a yield of 65%, but after repeating the reaction with this product a yield of 98% was obtained. The β-glucan tricarbanilate was soluble in 1,4-dioxane.

Characterization techniques

Light scattering

The technique of combined static and dynamic light scattering is a powerful method to determine absolute molecular weight and to characterize solution behaviour and molecular shapes of macromolecules (Burchard, 1985; Burchard & Richtering, 1989). Static light scattering measurements were performed in a modified and fully computer controlled SOFICA-light scattering apparatus. The combined static and dynamic light scattering measurements were carried out with an automatic photo-goniometer of ALV-Langen (Hessen, Germany) equipped with an Ar-Ion-LASER from Spectra-Physics (Darmstadt, Germany). All light scattering experiments were performed at angles between 30 and 150° in steps of 5° at a temperature of 20°C.

The refraction index increments were taken from the literature (Huglin, 1972) and expressed as a function of the solvent refractive index n_0 giving the equation:

$$dn/dc = -0.5678\, n_0 + 0.9072. \qquad (1)$$

The usual error of the light scattering method is approximately 3%; according to the difficult sample preparation we assessed an error between 5 and 8%.

Viscometry
Further investigations were carried out by viscometry with a Haake rotational viscometer CV 100 equipped with a Mooney–Ewart measuring unit (DIN 53 018) (Haake, Karlsruhe, Germany). In these experiments, concentration, solvent and pH were varied. Determination of the intrinsic viscosities $[\eta]$ and pH dependence was made at 20°C and a shear rate of 30/s.

Theory

Following the theory of chain flexibility it is possible to obtain detailed information on the macromolecular conformation from the angular dependence (shape factor) of the scattering intensity (Kuhn, 1934; Kratky & Porod, 1949; Cassasa, 1955; Holtzer, 1955; Koyama, 1973; Denkinger & Burchard, 1991). Koyama's theory was originally developed for worm-like, one-stranded chains. However, the same model can be efficiently applied also to double and triple helices (Burchard, 1988; Coviello *et al.*, 1986; Dentini, 1988). As confirmed by electron microscopy this theory was even applicable to non-ionic surfactants with sugar head-groups which formed long filamentous semi-rigid micelles where the mass per unit length could be determined (Denkinger *et al.*, 1989). In this work, a Cassasa–Holtzer plot was applied, where the $qP(q)M_w$ dependence on $u = qR_g$ is examined on the basis of Koyama's theory of semiflexible (worm-like) chains, where $q = (4\pi n_o/\lambda)$, $\sin(\theta/2)$ is the scattering vector and $P(q)$ the shape (or particle scattering) factor. The data in such a Cassasa-Holtzer plot exhibit a characteristic curve with a maximum (at $u = 1.41$ for monodisperse and $u = 1.71$ for polydisperse chains that obey the Schulz–Flory most probable distribution), and a plateau at large u values. From the ratio of maximum/plateau heights the number of statistically independent Kuhn segments N_k of a chain can be determined. The plateau value multiplied by the particle weight, M_w, gives the linear mass density (mass per unit length) $M_L = (M/L)$ where L is the contour length. The weight average contour length is found from M_L as $L_w = M_w/M_L$. Finally, the ratio L_w/N_k leads to the length l_k of a Kuhn segment ($l_k = 2a$, where a is the persistence length). The determination of the parameters L_w, M_L and l_k and the chain length polydispersity were made by a non-linear least square fit of the experimental data on the basis of the Koyama theory. The fitting programme developed in our laboratory (Dolega, 1990) could be run on most personal computers.

RESULTS

Highly diluted solutions

Static light scattering experiments allow the determination of the weight average molecular weight M_w, the radius of gyration R_g and the second osmotic virial coefficient A_2 by use of Debye's equation

$$Kc/R_\Theta = 1/M_w + 1/3(\langle s^2\rangle_z/M_w)q^2 + 2A_2c. \qquad (2)$$

In this equation, K indicates an optical contrast factor, which depends on the refractive index increment dn/dc, c is the concentration, R_Θ, Rayleigh ratio (scattering intensity) and q the value of the scattering vector defined as:

$$q = (4\pi/\lambda)\sin\Theta/2, \qquad (3)$$

with $\lambda = \lambda_0/n_0$ the wavelength of the light in a medium of refractive index n_0, and λ_0, the wavelength in vacuum. Figure 2 shows an example of a Zimm plot, where Kc/R_Θ is plotted versus $q^2 + kc$, with k as a freely chosen constant. The intersection point with the ordinate of the extrapolated lines to zero concentration and to zero scattering angle, respectively, leads to $1/M_w$. The slope of the angular dependence at $c = 0$ corresponds to the z-average mean square radius of gyration $\langle s^2\rangle_z$. To simplify notation, R_g is defined as the square root of $\langle s^2\rangle_z$. The second virial coefficient A_z is obtained from the slope of the other limiting line at $q^2 = 0$.

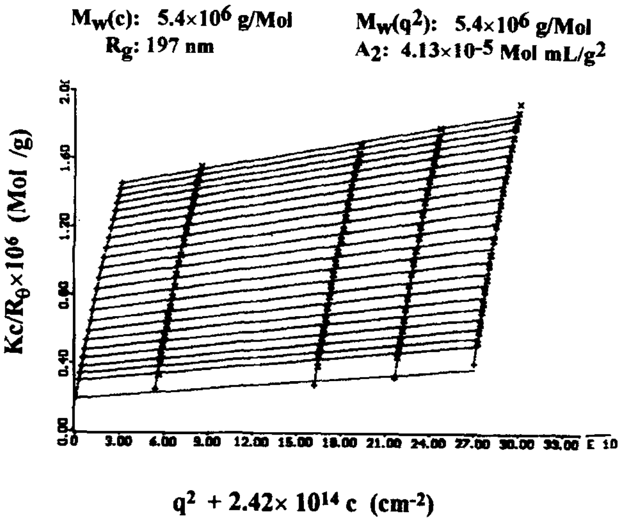

Fig. 2. Example of a Zimm plot from β-glucan, here dissolved in pure water at 20°C.

Table 1. Experimental results of static light scattering from highly diluted solutions of different solvents

Solvents	c (mg/ml)	M_w (g/mol)	x	R_g (nm)	A_2 (mol ml/g^2)	$[\eta]$ (ml/g)
Water	1.0530	5400×10^3	30.5	197	4.13×10^{-5}	219
90% DMSO	0.9632	3050×10^3	17.2	165	-8.75×10^{-6}	180
2 M GHCl	0.9782	2920×10^3	16.5	163	-1.62×10^{-4}	138
Cadoxen	1.1040	583×10^3	3.3	131	4.54×10^{-4}	–
Cuoxam I	1.0212	177×10^3	1	127	1.01×10^{-3}	–
Cuoxam II	0.9680	170×10^3	0.96	117	-3.72×10^{-3}	–

The temperature of measurement was always 20°C. c, concentration; M_w, molecular weight; x, degree of aggregation; R_g, radius of gyration; A_2, second osmotic virial coefficient; $[\eta]$, intrinsic viscosity.

Aqueous solutions

In a first series, 0.1% solutions of β-glucan in water with various additives were investigated. We used pure water, 90% DMSO and a 2 M solution of GHCl. The data are presented in Table 1. A strong dependence of particle weight on the mixed solvent composition was found as a result of aggregation.

Metal complexing solvents

The metal complexing solvents, cuoxam and cadoxen were used in a second series of experiments to minimize the interactions between β-glucan molecules. Both solvents are known to be good solubilizing reagents for cellulose. Cuoxam dissociated the aggregates and thus allowed determination of the weight average molecular weight of the unimer. For both samples, isolated from beer and the purchased one, M_w was found to be near $175{,}000 \pm 5000$ Da.

The experimental results are listed in Table 1. Figure 3 represents the plot of R_g vs $M_{w,\text{app}}$ in a double logarithmic plot. A surprisingly low exponent $a_{R_g} = 0.22$ is found: R_g increases only slightly while the particle weight increases by a factor of approximately 10^3. Such a low value cannot be explained even by spheres or spherical micelles which have an exponent of 0.33 (Tuzar & Kratochvil, 1993). Further details are provided in the Discussion.

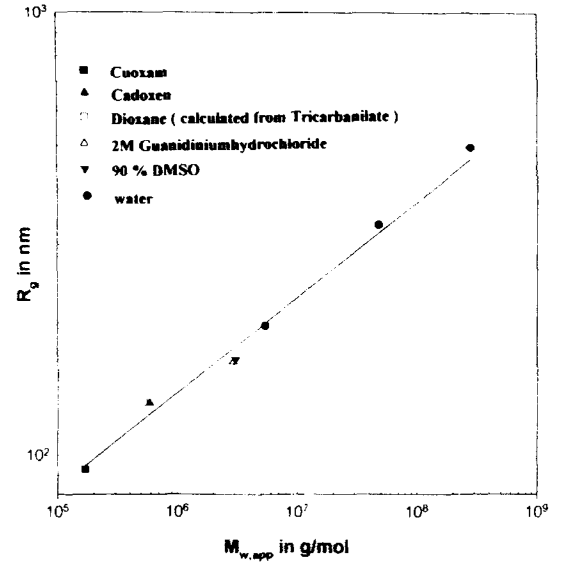

Fig. 3. Particle weight ($M_{w,\text{app}}$) dependence of the radius of gyration (Rg) of the β-glucan at 0.1% polysaccharide concentration in various aqueous and non aqueous solvents. (●), water obtained by dilution from stock solutions of various concentrations; (△) 2 M guanidinium hydrochloride: (▼), DMSO/H$_2$O = 90/10 (v/v); (■), cuoxam; (▲), cadoxen. The observed dependence follows the relationship $R_g = K\, M_{w,\text{app}}^{0.22}$. (Note, this dependence describes the behaviour of aggregates.)

Water/maltose mixtures as solvents

In a third series of experiments, maltose at different concentrations was included. This exerted an unexpected, strong and specific influence. Table 2 represents the experimental data. The results of static light scattering seem not to follow the same R_g versus M_w dependence as shown in Fig. 4. Similar behaviour was observed also by the independent rheological method (see Fig. 5) and confirms a change of structure near 5% maltose. Viscosities (η) at different concentrations were measured with a rotational viscometer, and the reduced viscosity was extrapolated to zero concentration to obtain the intrinsic viscosity $[\eta]$ using the Huggins (1942) equation:

$$\eta_{\text{red}} = \eta_{\text{sp}}/c\,[\eta] + k'[\eta]^2 c, \tag{4}$$

where η_{red} is the reduced viscosity, $\eta_{\text{sp}} = (\eta/\eta_0)\text{-}1$ the specific viscosity and k' the Huggins constant, with η_0 the solvent viscosity. Similar to the behaviour of the radius of gyration, a minimum of the reduced viscosity was found in 5% maltose/water mixtures.

Dynamic light scattering

Further investigations were carried out with dynamic light scattering from selected solutions. Here, a time correlation function is measured, and using the so-called cumulant fit the translational diffusion coefficient D_z is obtained.

$$\Gamma_1/q^2 = D_z(1 + k_D c)(1 + C R_g^2 q^2 - \ldots) \tag{5}$$

Table 2. Experimental data of highly diluted β-glucan in aqueous maltose solution measured at a concentration of approximately 1 mg/ml solvent mixtures

Solvents	c (mg/ml)	M_w (g/mol)	X	R_g (nm)	A_2 (mol ml/g^2)	$[\eta]$ (ml/g)
2% maltose	1.372	8650×10^3	48.9	209	1.12×10^{-4}	281
3% maltose	1.048	7870×10^3	44.5	191	9.66×10^{-5}	275
4% maltose	1.004	5690×10^3	32.1	151	8.67×10^{-5}	200
5% maltose	1.020	2870×10^3	16.2	146	5.20×10^{-5}	129
6% maltose	0.996	3150×10^3	17.8	126	6.74×10^{-5}	186
7% maltose	0.948	4340×10^3	24.5	137	9.76×10^{-5}	225
8% maltose	0.984	7985×10^3	45.1	147	1.11×10^{-4}	401
10% maltose	0.803	12000×10^3	67.8	247	1.87×10^{-4}	466

Abbreviations as in Table 1.

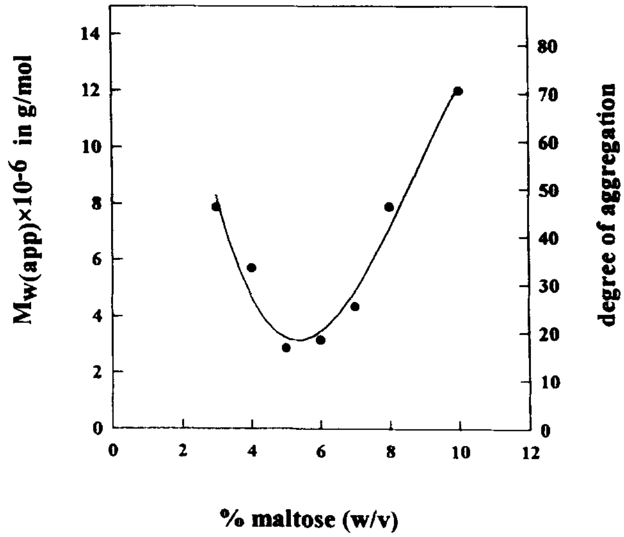

Fig. 4. Dependence of the apparent molar particle weight ($M_{w,app}$) on the aqueous maltose concentration. The degree of aggregation (X) is based on the molecular weight obtained in cuoxam.

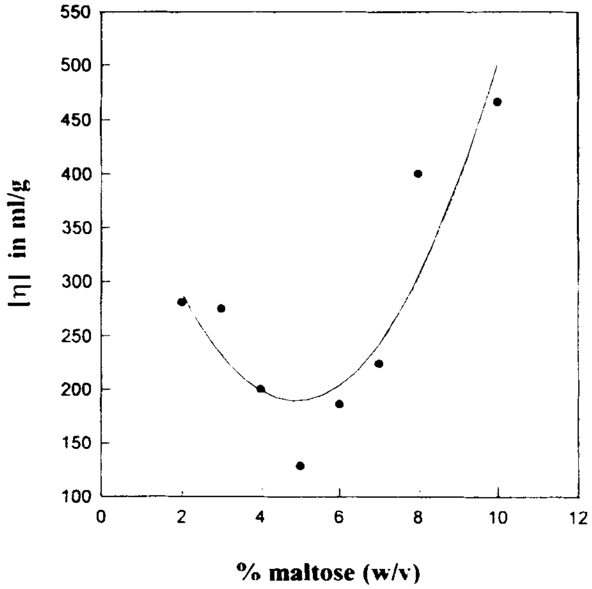

Fig. 5. Dependence of the intrinsic viscosity $[\eta]$ on the aqueous maltose concentration.

Γ_1 is the first cumulant, and q is given by equation (3) (Burchard *et al.*, 1980). This diffusion coefficient D_z is related to the dimensions of the coil and the solvent viscosity by the Stokes–Einstein relationship:

$$D_z = kT/6\pi\eta_0 R_h \qquad (6)$$

where R_h is an effective hydrodynamic radius. The solvent viscosity η_0 has been measured by capillary viscometry and is given in Table 3.

From a combination of static and dynamic light scattering, the ratio of $R_g/R_h = \rho$ can be found. This ρ-parameter is sensitive to the structure of particles in solution, and is thus a useful additional parameter for polymer and colloid characterization. A list of ρ-parameters is given in Burchard (1992). Table 3 lists the values of D_z, R_h and ρ found for the polysaccharide in the different solvent systems.

Table 3. Experimental results of dynamic light scattering

Solvent	D_z (cm^2/s)	η_0 (m Pa s)	R_h (nm)	$\rho = R_g/R_h$
Cuoxam I	2.50×10^{-8}	1.274	66	2.23
2 M GHCl	1.56×10^{-8}	1.525	83	1.96
6% maltose	1.38×10^{-8}	1.125	122	1.03
3% maltose	1.20×10^{-8}	1.073	178	1.07

D_z, diffusion coefficient; η_0, solvent viscosity; R_h, effective hydrodynamic radius; ρ, ρ-parameter.

β-glucan tricarbanilate

The β-glucan tricarbanilate was dissolved in dioxane. Measurements were made by static and dynamic light scattering, and the intrinsic viscosity was determined. The data are given in Table 4. The idea behind this experiment was that full derivatization of the OH-groups, responsible for H-bond formation, would remove all reasons for aggregation. However, three times as large a degree of polymerization was found with this derivative than obtained in cuoxam, but the results arising from this experiment are nonetheless interesting for discussion of the ρ-parameter and chain flexibility.

Table 4. Physico-chemical parameters of β-glucan tricarbanilate in 1,4-dioxane

c (mg/ml)	0.8563
M_w (g/mol)	583 000
R_g (nm)	131
A_2 (mol ml/g^2)	-2.51×10^{-4}
D_z (cm^2/s)	1.52×10^{-4}
η_0 (m Pas)	1.21
R_h (nm)	97.9
ρ	1.35
$[\eta]$ (ml/g)	122

Abbreviations as in Tables 1 and 3.

Intrinsic viscosities of β-glucan solutions with additive

Different aqueous solutions were used as solvents to investigate the influence of the solvent composition on the solution structure of β-glucan. The intrinsic viscosities in 90% DMSO, 2 M GHCl and different maltose concentrations were measured. Tables 1 and 2 contain the data. The relationship between $[\eta]$ and M_w followed power law behaviour i.e.

$$[\eta] = k\, M_w^{\,a} \qquad (7)$$

as is shown in Fig. 6. An exponent of $a_\eta = 0.725$ was found. Note, however, that M_w and $[\eta]$ are the properties of aggregates. No pH dependence was found in the pH range 2–11.

DISCUSSION

Structure of β-glucan in highly diluted aqueous solutions

To interpret light scattering data in terms of structural parameters, the molecular weight dependences of the parameters R_g, A_2, R_h, D_z and $[\eta]$ are usually examined (Table 5). These generally follow power laws of the type

$$Y = k_y M_w\, a_y \qquad (8)$$

where Y is the parameter involved.

For linear chains it is possible to apply scaling relationships (de Gennes, 1979) by which all exponents a_y can be derived from $v = a_y$. Such power laws indicate fractal objects with fractal dimension of $D_{\text{frac}} = 1/v$. Only values of $D_{\text{frac}} \leqslant 3$ represent physically reasonable structures. The exponents obtained from experimental data are compared in Table 6 with those obtained from the scaling laws. The very low value of $v = 0.22$ leads to an apparent fractal dimension of $D_{\text{frac}} = 4,5$ which cannot be explained by any geometrical architecture of the same universality class. Furthermore, the values for the exponents a_A and a_η do not fit the scaling relationships. These two failures give rise to the conclusion that the observed objects are not simple homologous polymer structures. Evidently, structural change takes place on aggregation. The degree of aggregation $x = M_w/M_{w0}$ can be calculated from the particle weights M_w in different solvents and from that of the monomer M_{w0} found in cuoxam.

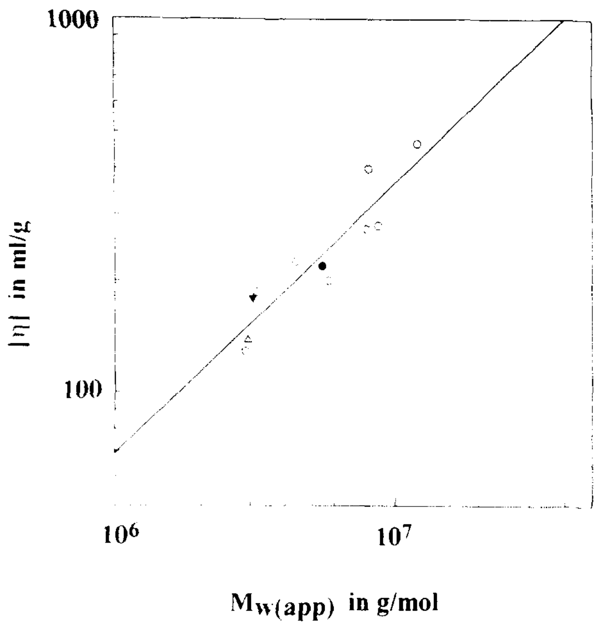

Fig. 6. Apparent molar particle weight ($M_{w,\text{app}}$) dependence of the intrinsic viscosity $[\eta]$ in all solvents and solvent mixtures applied. (The influence of maltose is not discernible.)

Table 5. Physico-chemical parameters of β-glucan at different concentrations in water

c (mg/ml)	1.053	10.98	21.60
M_{app} (g/mol)	5.4×10^6	47.4×10^6	277×10^6
R_g (nm)	197	333	497
A_2 (mol ml/g^2)	4.13×10^{-5}	1.1×10^{-6}	3.81×10^{-6}
D_z (cm^2/s)	1.19×10^{-8}	0.60×10^{-8}	
R_h (nm)	179	351	
ρ	1.10	0.93	

Abbreviations as in Tables 1 and 3.

Table 6. Exponents from plot log y versus log M_w for light scattering data and intrinsic viscosities compared with scaling laws and the resulting predictions for v

Exponents (measured)	Scaling laws	v (calculated)
$a_{R_g} = 0.22$	$a_{R_g} = v$	0.220
$a_{R_h} = 0.218$	$a_{R_h} = v$	0.218
$a_{D_z} = -0.218$	$a_{D_z} = -v$	0.218
$a_A = -0.86$	$a_A = 3v - 2$	0.380
$a_\eta = 0.725$	$a_\eta = 3v - 1$	0.575

Abbreviations as in Tables 1 and 3.

The surprisingly low exponent a_{Rg} indicates a large increase of particle weight without appreciable growth of R_g. Thus, random aggregation can be discarded, since according to percolation theory, these clusters grow in molecular mass and size with a fractal dimension of $D_{\text{frac}} = 2.0$–2.5 (de Gennes, 1976; Stauffer, 1976). There remain two models which have to be taken into consideration. One is star-shaped spherical micelles, which are usually observed with block copolymers (Tuzar & Kratochvil, 1993). Since in this case a closed association equilibrium is reached with a defined aggregation number this model has to be excluded for the present system.

The other structure is the fringed micelle type, where side-to-side aggregation of chains occurs. In the outer region of the micelle the chain sections keep their flexibility while within the 'stem' the chain sections become stiffened. Figure 7 shows a schematic representation of such fringed micelles at two different aggregation stages. Clearly, there will be only a weak increase in the dimensions when more and more chains are laterally aligned, once a fairly rigid 'stem' has been formed. A modification of the model of the fringed micelle is also depicted on the right of Fig. 7c, which differs from those on the left by a growth in thickness *and* in length as molar mass is increased.

To examine the applicability of this model to β-glucan, chain stiffness was determined on the basis of the Koyama theory for worm-like chains. A Cassasa–Holtzer plot was applied to β-glucan dissolved in water and 2 M GHCl and fitted according to the prescription as described in the theoretical section. The characteristic parameters are shown in Table 7. The number of statistically independent Kuhn segments, N_k, were found to vary only insignificantly but l_k and L_w increase when aggregation extends. This fact indicates micelle growth in thickness *and* in length. Hence, β-glucan in highly diluted aqueous solutions forms a type of fringed micelle in which the aggregated sections appear to be not exactly matched with respect to their lengths but the chains forming the stem are partly staggered. The Kuhn segment lengths l_k (twice the persistence length) are extremely large and indicate a considerable reduction in the internal flexibility of the aggregated structure.

Table 7. Worm-like chain parameters derived from Cassasa–Holtzer plots for β-glucan dissolved in 2 M GHCl, water and maltose/water mixtures

Solvent	l_k (nm)	L (nm)	N_k	M_L (g/mol/nm)	n
2 M GHCl	144	841	5.8	3500	11.1
water	166	1100	6.6	4930	15.7
3% maltose	185	1290	7.0	5980	19.0
5% maltose	98	658	5.6	3900	12.4
6% maltose	129	722	6.7	4780	15.2
8% maltose	114	1210	10.6	6360	20.2
10% maltose	148	1580	10.6	8440	27.7

The data were obtained by non-linear least square fits with the Koyama theory for semiflexible structures. l_k, Kuhn segment length; L, contour length, N_k, number of Kuhn segments per particle, M_L, linear mass density, $n = M_L/M_{Lo}$, multiplicity of aggregated structures.

Structure sensitive parameter ρ

Similar conclusions to those drawn from the data for worm-like chains can be drawn from the ρ-parameter. For the β-glucan in different aqueous solutions this structure sensitive ρ-parameter at first decreases drastically with increasing particle weight but then remains virtually constant when $M_w > 10^6$ Da (Fig. 8). A value of $\rho \cong 1.0$ corresponds to star-branched structures (Burchard & Richtering, 1989) and would be in agreement with the suggested fringed micelle in a highly aggregated state (Fig. 7c).

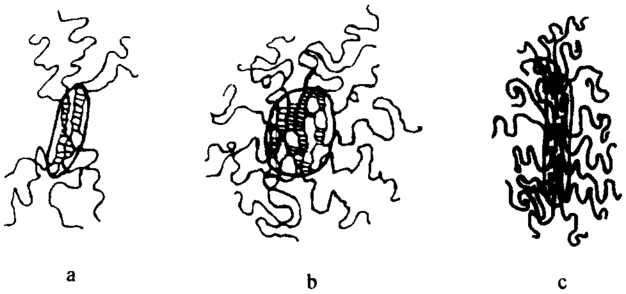

Fig. 7. Fringed micelles at two different aggregation stages (a, b) and a modified fringed micelle (c).

Structure of β-glucan dissolved in maltose solvent mixtures

Minimum aggregation was obtained by light scattering and viscometry for the β-glucan in maltose/water of approximately 5% (w/v) maltose content. This behaviour provides evidence for disaggregation that is connected with a structural change that would correspond to an increased chain flexibility. This conjecture was checked by Cassasa–Holtzer plots in connection with the fitting procedure according to the Koyama theory. The plots and fitted curves are shown in Fig. 9

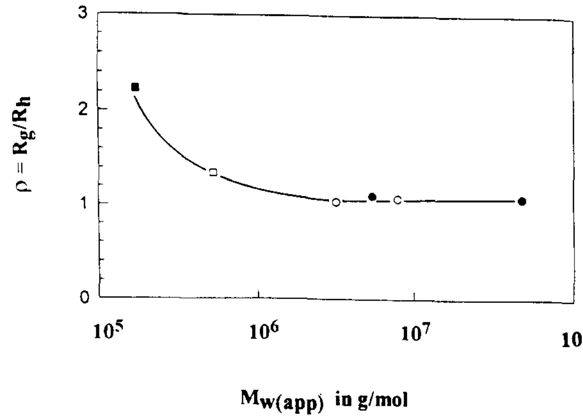

Fig. 8. Particle weight dependence ($M_{w,app}$) of the parameter $\rho = R_g/R_h$ for glucan dissolved in various solvents as shown in Fig. 3. The radius of gyration, R_g, was measured by static light scattering and the hydrodynamic radius, R_h, by dynamic light scattering.

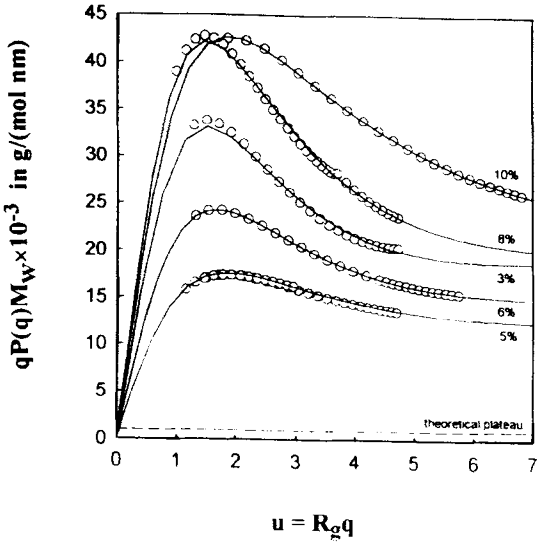

Fig. 9. Cassasa–Holtzer plot for 0.1% glucan solution dissolved in aqueous maltose solutions of the indicated percentage. The Cassasa–Holtzer plot allows determination of the chain stiffness of semiflexible chains applying a fitting programme derived from the Koyama theory, N_K, is the number of statistically independent Kuhn segments per chain. For further details see Table 7.

and the data are collected in Table 7. Indeed, a maximum flexibility of the β-glucan particles is now found as documented by the minima of l_k, L, N_k and M_L in the 5% maltose solution. Evidently, the aggregation is partially broken up as a result of a competitive inhibition mechanism (Rees *et al.*, 1982).

Table 7 also reveals another interesting property of the aggregates described by n, the number of lateraly aggregated strands, which was calculated from the linear mass density according to equation (9),

$$n = M_{L\,(\mathrm{exp})}/M_{L\,(\mathrm{single\ strand})} \tag{9}$$

where the linear mass density of the single chain was calculated from the averaged molar mass and length of a repeating unit ($l_0 = 2.7 \times 0.515\,\mathrm{nm} + 0.48\,\mathrm{nm} = 1.9\,\mathrm{nm}$ and $M_0 = 3.7 \times 160$ g/mol = 592 g/mol). This number n varies between 11 and 28 with a peak at approximately 14.7 ± 5 with the exception of the two highest maltose concentrations. Thus, the thickness remains in the same range, while the total aggregation number x shows a stronger variation. Here again a growth in length to some extent has to be calculated. Thus, taking all the facts together, we come to a suggested model that very much resembles the modified fringed micelle of Fig. 7c.

The data for η and R_g in pure water do not fall on the corresponding parabola of the maltose data. However, the solution preparation for β-glucan in pure water was found to depend strongly on the pretreatment (temperature, concentration, pressure) when autoclaved. This point becomes clear from Fig. 3, where we show three different preparations of aqueous solutions. To emphasize this point, the reproducibility of individual measurements is high, but depends very strongly on the history of the sample.

Comparison with the β-glucan from oat aleurone

The aggregation behaviour of oat aleurone β-glucan, as investigated by Vårum *et al.* (1992), shows significantly different behaviour from the β-glucan studied in this paper.

(1) Vårum *et al.* found complete dissociation into monomers on dilution to zero concentration. Such dissociation could not be achieved with our material.

(2) The apparent molecular weight increased approximately to an aggregation number of $x = 4$–5, where in our case the aggregation number varied between 17 and 70.

(3) The oat aleurone β-glucan pH was found to be 4.85–4.06 when the concentration was increased, which indicates negative charges on the chain. No polyelectrolyte character was found with the barley β-glucan. Because of the repulsive interactions, the aggregation tendency is strongly reduced and allows complete dissociation on dilution. Furthermore, the block length of the oat β-glucan is shorter than in the barley β-glucan as described by Dais and Perlin (1982) and Wood *et al.* (1991, 1994), and block lengths of more than 9 following β-(1,4)-bonds are expected to be fully insoluble in water (Wood *et al.*, 1991).

Vårum *et al.* tried to fit their data to a spherical micelle model. However, their calculations completely neglected the intermolecular volume interactions, which were shown to have an significant influence on the apparent molecular weight (Burchard, 1988). Taking account of these interactions, the true molecular weight increases further with increasing concentration. Therefore, their results indicate an *open* association rather than a *closed* association. Regarding the difference in chain stiffness it can be said that the initial aggregation up to $x = 5$ does not substantially change the flexibility of the chains. Recent experiments with hemicellulose (Ebringerova *et al.*, 1994) provided clear evidence that chain stiffening occurs when at least six chains have aggregated side-by-side.

These facts draw us to the conclusion that the aggregation process is basically the same for both β-glucans, and the main difference consists of the electrical charge, which shifts the equilibrium more towards dissociation.

The structure of β-glucan tricarbanilate in dioxan

The β-glucan tricarbanilate was investigated and a molecular weight of $M_w = 583{,}000$ Da was found which still gave a degree of aggregation of $x = 3.33$ when the molecular weight of the monomer found in cuoxam is taken. From this, it might be concluded that an aggregation of three β-glucan carbanilate chains is thermodynamically favoured. The value of $\rho = 1.35$ seems to indicate linear flexible chain behaviour in a poor solvent, but fitting of the Cassasa–Holtzer plots gave data of $l_k = 274$ nm, $L_w = 595$ nm, $N_k = 2{,}17$ and $M_w/L_w = 2732$ g/(mol nm) which demonstrate a very high rigidity. These results are not necessarily in contradiction, because chain stiffness increases the ρ-parameter (Schmidt, 1984) but lateral aggregation lowers it again. Hence, the observed ρ-parameter is the result of two partially counter balancing effects. Calculation of the laterally aggregated strands, n, according to equation (9) leads to $n = 2.9$, where $M_0 = 3.7 \times 160$ gmol $\times 3 = 1776$ g/mol. This value indicates a strictly lateral aggregation in the case of tricarbanilate. We added these results since a full substitution (polymer analogous deviation (Staudinger, 1932)) has been considered as the most convincing technique for distinguishing covalent from non-covalent bonds. The present example shows now, that even the fully derivatized sample can undergo a stable aggregation.

CONCLUSION

In aqueous solvents, β-D-(1,3)(1,4)-glucans are not molecularly dispersed. The aggregation number strongly depends on the solvent composition and temperature. A minimum of aggregation is found in 5% aqueous maltose solutions. The effect is interpreted as a preferential binding of maltose which partly breaks up the aggregated clusters. Simultaneously, the remarkable cluster rigidity is decreased. The behaviour is consistently explained by a modified fringed micelle structure for the clusters. Full molecular dissolution is obtained only in cuoxam, a copper complexing solvent known to dissolve the β-(1,4)-glucan cellulose.

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