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A COMPARISON OF THE SIZE AND SHAPE OF β-LIMIT DEXTRIN AND AMYLOPECTIN USING PULSED FIELD-GRADIENT NUCLEAR MAGNETIC RESONANCE AND ANALYTICAL ULTRACENTRIFUGATION

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ABSTRACT

The size and shape of β-limit dextrin have been investigated by using pulsed, field-gradient nuclear magnetic resonance and analytical ultracentrifugation. In addition, the β-limit dextrin has been compared with the amylopectin from which it was derived by enzymic hydrolysis. When measuring size and shape, dimethyl sulfoxide was used as the solvent, in order to avoid problems of polymer aggregation. The results suggest that β-limit dextrin is an oblate ellipsoid with an axial ratio of ~5:1, and the corresponding amylopectin molecule is even flatter. This indicates that the linear segments beyond the final branch-points of amylopectin lie in the plane of its branched core. The study also demonstrated that the density of packing of polymer chains in this branched core is much greater than at the periphery of amylopectin, and that the latter region is the location of the great majority of the nonreducing chains cleaved by beta amylase. Furthermore, the different sized molecules in amylopectin samples appear to undergo the same degree of degradation by this enzyme.

INTRODUCTION

Various methods may be used to assess the size and shape of polymers in solution. In the case of pulsed field-gradient nuclear magnetic resonance (p.f.g.-n.m.r.) and analytical centrifugation, information obtained by one technique complements that obtained by the other. The present paper describes an investigation of the size and shape of β-limit dextrin using these two methods. In addition, a comparison was made between β-limit dextrin and the amylopectin from which it is derived by enzymic hydrolysis. The comparison was undertaken in an attempt to gain information concerning the spatial distribution of monomer units and the location of the nonreducing chains in amylopectin. The results of previous work had

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suggested that the distribution density of monomer decreases continuously in a radial direction and that the nonreducing chains are at the periphery of the molecule[1].

The fundamental chemical structure of the amylopectin molecule from which β-limit dextrin is derived is that of an α-(1→4)-linked D-glucose polymer having some 4–5% of α-(1→6) branch points. Evidence to date suggests that a racemose or cluster type of model reflects the properties of amylopectin better than any other structure. The main feature of the model is that the branch points are arranged in tiers or clusters, and are not distributed uniformly throughout the macromolecule. When the enzyme beta amylase acts on amylopectin, the next-to-last glycosidic bond from the nonreducing end of the polymer chain is cleaved to release maltose. The action of the enzyme is blocked by α-(1→6) branch linkages. Prolonged action of beta amylase results in the formation of β-limit dextrin that contains ~40% of the D-glucose units and all of the branching of the original amylopectin molecule[2–5].

Relatively little is known of the size and shape of β-limit dextrin. However, the size and shape of the parent amylopectin molecule in dimethyl sulfoxide (Me_2SO) as the solvent have been studied by p.f.g.-n.m.r.[6,7] and analytical ultracentrifugation[8]. The use of Me_2SO avoids problems arising from the tendency of amylopectin to aggregate in water. P.f.g.-n.m.r. measurements suggest that, in Me_2SO, wheat-starch amylopectin is an oblate ellipsoid having a semi major to semi minor axis ratio (a/b) in excess of 6. M_w is estimated very approximately to be of the order of 10^6. The amylopectin weight-averaged, dilute-limit, self-diffusion coefficient measured by p.f.g.-n.m.r. can be shown to agree with the corresponding z-average diffusion coefficient measured by quasi-elastic light-scattering[9], provided that the M_w/M_n ratio for amylopectin is ~300. Independent theoretical and experimental evidence justifies this high molar mass ratio[10]. Ultracentrifuge measurements indicate a larger M_w (~10^7) and a higher semi-axial ratio (~30) than suggested by p.f.g.-n.m.r. However, the theoretical foundations upon which the analytical ultracentrifuge and p.f.g.-n.m.r. analyses are based differ, and such numerical discrepancies are not unexpected.

The application of p.f.g.-n.m.r. to polymer systems has been reviewed[7]. Only salient points of the approach are considered here. P.f.g.-n.m.r. provides a means of measuring both solvent and polymer self-diffusion coefficients. The diffusion of solvent is monitored by exploiting its overwhelmingly powerful proton n.m.r. signal strength, whereas, when the polymer signal is of interest, a perdeuterated solvent is needed.

The dependence of solvent diffusion on dilute polymer concentration may be interpreted by using the modified Wang model[11].

$$D^{solv} = D_0^{solv}\,[1 - w\{(\bar{\alpha} - 1)(V_p d_0 + h) + h\}], \qquad (1)$$

where w is the macromolecule weight fraction, V_p the apparent macromolecular specific volume, d_0 the density of pure solvent, h the polymer solvation coefficient, D^{solv} the solvent diffusion coefficient, and D_0^{solv} the pure solvent diffusion coefficient. $\bar{\alpha}$ is related to the axial ratio (a/b) of the ellipsoid of revolution that is used as a hydrodynamic model of polymer shape.

Next, the polymer self-diffusion coefficient at infinite dilution, D_0, may be analyzed by using the Stokes–Einstein relationship, to give the Stokes radius, R_D, of the macromolecule.

$$D_0 = k_B T/6\pi\eta R_D, \tag{2}$$

where η is the solvent viscosity. For a high polydispersity system, the apparent polymer self-diffusion coefficient that is measured must be corrected to yield the true value of $D_0(M_w)$. For a nonspherical shape, such as an ellipsoid of revolution,

$$R_D = R_0 f_s, \tag{3}$$

where R_0 is the radius $(a^2b)^{1/3}$ of a sphere of equal volume and f_s is a known function of the ratio a/b. Hence, once a/b is known from the Wang experiment, the volume term (a^2b) may be established from D_0 measurements. Finally, the concentration dependence of the solute self-diffusion coefficient may be analyzed[7], to give an independent estimate of a/b.

The procedure used in the present study to evaluate ultracentrifugation data has been described in detail[8], and hence only an outline of the approach is given here. For a monodisperse system, the dilute limit sedimentation coefficient, s_0, is related to the polymer molar mass by the Svedberg equation,

$$M = \frac{s_0 RT}{D_0(1 - \upsilon d_0)}, \tag{4}$$

where υ is the partial specific volume. However, for a polydisperse system, the method has theoretical shortcomings, because, in this case, s_0 and D_0 are weight-averaged coefficients which are determined separately, albeit in solutions of identical composition. Strictly, it is the weight-averaged ratio $(s/D)_0$ that should be employed. In the present instance, the Svedberg equation is used to provide an approximate value of M from which the polymer dimensions may be estimated.

Combining the Svedberg and Stokes–Einstein relationship gives an expression for F, a structural parameter that is a known function of a/b.

$$F = \frac{s_0\eta}{(1 - \upsilon d_0)}\left[\frac{162\pi^2 N^2 \upsilon}{M^2}\right]^{1/3}, \tag{5}$$

where N is the Avogadro number.

The actual molecular dimensions may then be estimated.

$$a = \left(\frac{3Mv}{4\pi N}\right)^{1/3} \left(\frac{a}{b}\right)^{1/3} \qquad (6)$$

MATERIALS AND METHODS

Preparation of starch, amylopectin, and β-limit dextrin. — Starch was extracted from wheat-grain *cv Aotea* as described previously[8]. Amylopectin was fractionated from the starch by classical methods[4], and preserved by freeze-drying. Potentiometric iodine-titration curves[4] showed the polymer to be of high purity, with an iodine-binding capacity of 0.3%. Crystalline beta amylase enzyme isolated from sweet potato was purchased from Sigma. The purity of the enzyme was checked by using Amylose Azure[12]. β-Limit dextrin was prepared by using standard methods[4]. The extent of the reaction was determined by removing maltose by dialysis and measuring the residual polymer. The concentration of polymer was determined by using the phenol–sulfuric method[13] and by enzymic assay (amyloglucosidase)[14].

Solutions of β-limit dextrin, or amylopectin, in Me_2SO were equilibrated for 10 days in a nitrogen atmosphere before measurements were made.

P.f.g.-n.m.r. measurements. — The self-diffusion experiments were performed by using 60-MHz proton spin echoes from a JEOL FX60 NMR spectrometer interfaced to a pulsed field-gradient system as described previously[6]. Measurements were made at 27°.

Analytical ultracentrifuge techniques. — The sedimentation experiments were conducted by using a Beckman Model E analytical ultracentrifuge as described elsewhere[8].

Partial specific volume. — Partial specific volumes were determined with an Anton–Paar precision system DMA 60/602 density meter. Data were analyzed by the procedure of Kratky *et al.*[15].

RESULTS AND DISCUSSION

Fig. 1 shows the dependence of solvent (Me_2SO) self-diffusion on polymer concentration for amylopectin and its β-limit dextrin. The data may be compared with those obtained[7] for glycogen, which is known to be spherical. The similarity of the amylopectin and β-limit dextrin data immediately suggests that both structures have similar axial ratios. A quantitative interpretation using the Wang equation requires an estimate of the solvation factor h. It had been shown[6] that $h \leq 2.0$ for Me_2SO solutions of polysaccharides, so that the data of Fig. 1 yield the following.

For amylopectin in Me_2SO, $\alpha \geq 2.0 \Rightarrow a/b \geq 4.5$ (oblate ellipsoid).

For the β-limit dextrin in Me_2SO, $\alpha \geq 1.9 \Rightarrow a/b \geq 4.0$ (oblate ellipsoid).

It is noteworthy that the Wang equation analysis of the solvent self-diffusion data is consistent only with an oblate structure, as, for prolate ellipsoids, $\alpha \leq 1.67$.

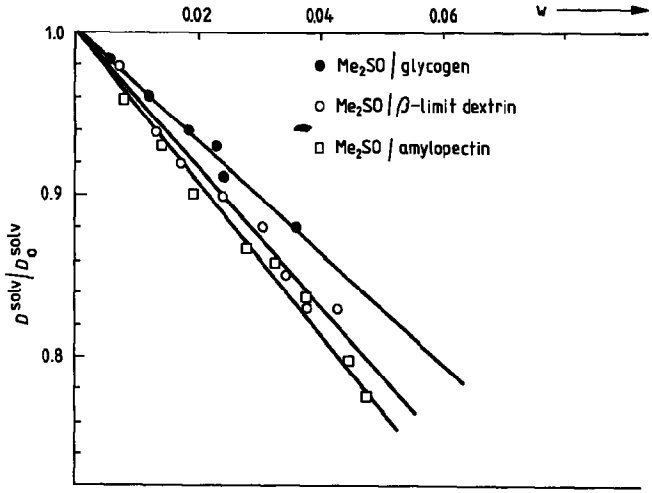

Fig. 1. Dependence of Me_2SO self-diffusion on amylopectin and β-limit dextrin. [The similar slopes indicate similar shape factors. Comparison with the corresponding glycogen data shows the influence of molecular shape on the solvent self-diffusion.]

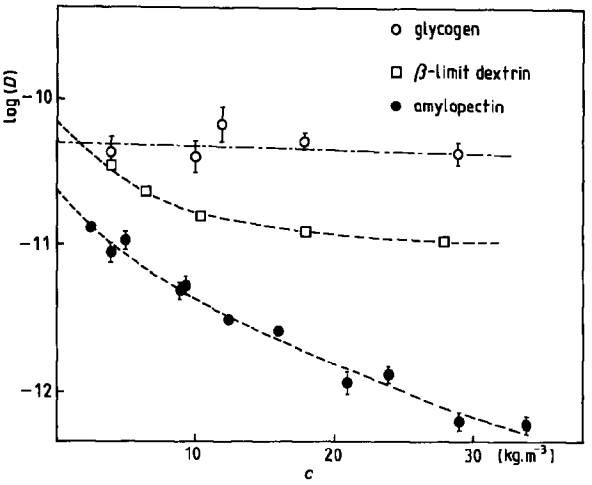

Fig. 2. Amylopectin and β-limit dextrin self-diffusion *vs.* concentration for Me_2SO solution. [The data are plotted as log (D) *vs.* c, and D_0^{eff} values are found by extrapolation. The glycogen data[7] are shown for comparison.]

The respective amylopectin and β-limit dextrin polymer self-diffusion are shown in Fig. 2, along with that of the spherical polysaccharide glycogen. Again, the amylopectin and glycogen exhibit different behavior, the strong concentration-dependence of the amylopectin self-diffusion being consistent with a highly nonspherical conformation. In contrast with the solvent behavior, the polymer self-diffusion is more sensitive to differences between the amylopectin and its β-limit dextrin, and it is apparent that both the diffusive concentration-dependence and

TABLE I

SELF-DIFFUSION OF AMYLOPECTIN AND β-LIMIT DEXTRIN

Polysaccharide	M_w/M_n	$D_0 eff$ $(10^{-11}\ m^2.s^{-1})$	$D_0(M_w)$ $(10^{-11}\ m^2.s^{-1})$	R_D *(nm)*
Amylopectin	~300	2.8(7)	0.7(1)	22(3)
β-Limit dextrin	assumed ~300	6.6(8)	1.9(2)	8(1)

the infinite-dilution, self-diffusion coefficients differ. The results are summarized in Table I, where errors are given in parentheses and correspond to standard deviations in the least significant digits. Effective D_0 values are corrected for polydispersity ($M_w/M_n = 300$ (ref. 10)), to give $D_0(M_w)$ following the method of ref. 6.

The mean hydrodynamic radii of the amylopectin and β-limit dextrin are 22(3) and 8(1) nm respectively. In order to compare hydrodynamic volumes, $(a^2b)^{1/3}$, the respective shape factor influences, f_s, in Eq. *3* must be determined. In fact, f_s is weakly dependent[16] on a/b with $1.0 \leq f_s \leq 2.0$ for $1 \leq a/b \leq 40$. The a/b ratios obtained from the solvent-diffusion experiment imply that the respective f_s values are the same within 1%. On that basis, a volume ratio of 18(7) is suggested.

The sedimentation data yield infinite dilution s_0 values for amylopectin and its β-limit dextrin of 60(10) and 31(5) S, respectively. Using the $D_0(M_w)$ values obtained from p.f.g.-n.m.r., s_0 values from sedimentation, and v values of 0.58(1) $cm^3.g^{-1}$ for both polymers, the Svedberg equation implies the following molar masses.

For amylopectin, $M_w = 6(2) \times 10^6$. For β-limit dextrin, $M_w = 1.2(1) \times 10^6$. According to this analysis, the beta amylase removes 80(10)% of the amylopectin mass. This compares with the value of 64(4)% obtained directly *via* enzyme analysis. Given the high polydispersity of the polymer, the agreement is quite satisfactory and gives credence to the analysis this far.

The apparent mass ratios of between 3 and 5 differ markedly from the volume ratio determined from $D_0(M_w)$ data and suggest that the polymer densities in the amylopectin and β-limit dextrin are quite different. We have already remarked that this interpretation does depend on the respective a/b ratios and consequent f_s values.

An independent estimate of a/b for each polymer can be obtained from the absolute s_0 and molar mass values as shown by Eq. *5*. Using such an analysis we find the following ratios. For amylopectin, $a/b = 28(10)$. For β-limit dextrin, $a/b = 6(1)$.

Although a greater amylopectin a/b ratio is observed, in qualitative agreement with the Wang equation analysis, the difference is more marked in the sedimenation experiment. This may reflect conformational differences resulting from ultracentrifugation, but nonexistent pressure-dependence[8] would appear to

exclude this possibility. Using the sedimentation a/b values and corresponding f_s ratio of 1.3, the ratio of the amylopectin to β-limit dextrin hydrodynamic volumes may be reanalyzed to yield 40(20). Using the extreme mass and volume ratios obtained here, the density ratio of the amylopectin and β-limit dextrin lies between 4 and 13.

The data presented here are consistent with a polymer chain density in the β-limit dextrin that is much higher than in the untreated amylopectin. Both polymer structures, however, are similarly oblate, with amylopectin somewhat the flatter. These observations are consistent with the schematic, structural model in which the linear segments beyond the final branch-points lie in the plane of branched core. The hydrodynamic volume of amylopectin includes a significant portion of the outer, linear-chain segment, because of the well known disturbance[17] to solvent motion in the vicinity of the polymer chains. The beta amylase removes these linear segments up to the first branch point. The remaining dextrin is highly branched and, in consequence, the monomer density is greater. This is in contrast to glycogen, where the density of packing of polymer chains increases from the core towards the periphery of the molecule[18]. The similarity of the a/b ratios of β-limit and amylopectin clearly indicates that the linear segments beyond the final branch points are arranged in a planar conformation. This is a somewhat surprising result, and suggests that the outer linear chains do not behave as random coils but instead experience a long-range attractive interaction which causes them to condense in a two-dimensional layer. This may well be an influence of the low Me_2SO solvent quality previously observed[7]. Alternatively, some hydrogen bonding may occur between the chains.

Polydispersity has a large effect on p.f.g.-n.m.r. measurements of polymer self-diffusion. With an M_w/M_n ratio of ~300, as occurs with amylopectin, there is about a ten-fold difference between D_0 measured and the corrected $D_0(M_w)$. The M_w/M_n ratio for β-limit dextrin is uncertain, but the data from the present study suggest that the ratio is similar to that for amylopectin. If this were not the case, the M_w ratio of β-limit dextrin to amylopectin, which has been calculated by using the $D_0(M_w)$ figure for β-limit dextrin, would not be of the order expected from direct measurements of the maltose released by beta amylase. The $D_0(M_w)$ figure for β-limit dextrin assumes an M_w/M_n of 300. Hence, differences in the degree of degradation of different-sized molecules in an amylopectin system appear unlikely. This agrees with previous light-scattering studies of corn amylopectin and its beta amylase limit dextrin[1].

The present study also suggests that the great majority of the polymer removed by beta amylase is at the periphery of amylopectin. This is evident from the relatively large difference in hydrodynamic radii between β-limit dextrin and the amylopectin from which it is derived. Nonreducing chains do not appear to reside in the interior of amylopectin close to the center of gravity of the molecule, and again, this agrees with the results of light-scattering studies[1].

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