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THE SIZE AND SHAPE OF AMYLOPECTIN: A STUDY USING ANALYTICAL ULTRACENTRIFUGATION

JOHN LELIEVRE, JAMES A. LEWIS*, AND KAREN MARSDEN
Food Technology Department, Massey University, Palmerston North (New Zealand)

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ABSTRACT

Starch was isolated from a range of wheat cultivars grown under various conditions. The starches were known to produce pastes having contrasting rheological properties when heated. Solutions of the starch in dimethyl sulphoxide were investigated by analytical ultracentrifugation. Sedimentation coefficients and normalized weight–frequency distributions of limiting sedimentation-coefficients were determined. The sedimentation-coefficient data confirmed the weight-average molar mass of amylopectin in dimethyl sulphoxide to be $\sim 10^7$, irrespective of the source of the starch. The sedimentation-coefficient values were also combined with diffusion-coefficient data and evaluated according to hydrodynamic theory, this indicated that amylopectin has a flat-sheet or disc-like structure with semi-major and semi-minor axes of 45 and 1.2 nm, respectively. The distributions of sedimentation coefficients of the amylopectin samples were all similar, being unimodal, symmetrical, and having peak-width half-heights of ~60 *S*. The amylopectin obtained after removal of amylose by chemical fractionation was shown to be representative of the total amylopectin in starch.

INTRODUCTION

Starch granules consist of ~99% w/w hydrated polysaccharide. In most plant species, the major component is amylopectin; the remaining polymer is mainly amylose, a virtually linear (1→4)-α-D-glucan. Although amylose has been studied extensively, many features of amylopectin are incompletely understood[1–3].

The arrangement of the branches within amylopectin is uncertain. Enzymic studies have led to models of the polymer based on trichitic, racemose, and branched double-helical structures. None of these models is fully accepted. In any event, amylopectin molecules vary with respect to the number of branches per molecule, the length of branches, and the arrangement of branches into a pattern[4]. The molar masses and sizes of amylopectin structures in aqueous solution have also been investigated. Chemical end-group procedures[5] and osmotic-pressure measure-

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*Physics and Biophysics Department, Massey University, Palmerston North, New Zealand.

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ments[6] yield values of M_n of ~3×10^5, whereas light-scattering techniques indicate[7,8] M_w to be ~4×10^8. Various sources of amylopectin have been used in these molar-mass studies, and it is not clear whether this influences the results so obtained. The ratio of M_w/M_n for a given sample of amylopectin has been reported to be 300, suggesting a wide range of molecular sizes to be present. There is some theoretical justification for a high M_w/M_n ratio, based on random-condensation treatments of branched-polymer formation[9,10].

One of the problems in studying the size and shape of amylopectin in aqueous solution is the tendency of the polymer to aggregate. For this reason a number of investigators have used dimethyl sulphoxide, a more-powerful solvent in which aggregation is avoided. Measurement of the self-diffusion coefficient of amylopectin in Me_2SO using quasi-elastic light scattering[11] gives a dilute-limit diffusion coefficient of 8×10^{-13} $m^2 s^{-1}$. The corresponding hydrodynamic radius is considerably lower than that found in aqueous systems. Another method of measuring self-diffusion coefficients is by pulsed field-gradient nuclear magnetic resonance (p.f.g.–n.m.r.)[12]. This is powerful method for studying macromolecules as it may be used to probe both solvent and polymer diffusion-rates. Both of these are influenced by macromolecular size and shape. Such measurements demonstrate that molecules of wheat-starch amylopectin in Me_2SO are highly planar and behave as oblate ellipsoids having a semi-major axis, a, of 22 nm, and semi-minor axis, b, of 1.2 nm. M_w is of the order of 10^6. In contrast, the p.f.g.–n.m.r. technique shows that, in aqueous systems, amylopectin is aggregated into a more-spherical shape and has a volume some 400 times larger than the single molecule[13].

The present paper describes further studies on starch polymers in Me_2SO, principally by analytical centrifugation. Sedimentation coefficients and distributions of weight-average sedimentation coefficients were measured. The investigations, which were mainly concerned with amylopectin, had a number of objectives. The first objective was to measure the sedimentation coefficient of an amylopectin sample in Me_2SO and compare the M_w value so obtained with the p.f.g.–n.m.r. results published previously[13]. The second objective was to compare the molar mass of amylopectin extracted by classical chemical fractionation[3] with that of amylopectin in unfractionated starch, to ensure that the amylopectin obtained after chemical removal of amylose is representative of the total amylopectin in the starch. Thirdly, a comparison of the molar masses of starch polymers from a range of wheat cultivars grown under various conditions was made; preliminary evidence suggests that, within any one botanical species, there is some variation in the molecular size of amylopectin[3]. The starches from the different wheat cultivars studied form pastes with contrasting rheological properties[14]. Such pastes, which are of industrial interest, consist of colloidal gel particles dispersed in a continuous phase. The gel particles are swollen starch-granule fragments, the volumes of which determine the rheological properties[14–17]. The possibility that variations in swelling capacity between starches from different wheat cultivars may be linked to differences in the molar masses of the starches was investigated. Finally the dimensions and shape of amylopectin molecules were determined from analytical ultracentrifuge data, using hydrodynamic theory.

MATERIALS AND METHODS

Preparation of starch and of amylopectin. — Wheat grains from different cultivars, grown under various conditions, were obtained from the New Zealand Wheat Research Institute, Christchurch. Starch was isolated by the standard procedure of Meredith *et al.*[18] which uses mildly acidic conditions to inactivate amylases in the grain. To check for any concomitant acid depolymerization, control samples were isolated by the non-acidic method of Banks and Greenwood[3]. The starches extracted by the two procedures were then dissolved in Me_2SO and viscosity measurements made as described previously[11]. The viscosity results so obtained showed no evidence of polymer degradation.

Amylopectin was isolated by classical fractionation methods[3] and freeze-dried. Potentiometric iodine-titration curves showed[3] the polymer to be of high purity, with an iodine-binding capacity of 0.3%.

Solutions of starch or amylopectin in Me_2SO were prepared as described previously[11].

Analytical ultracentrifuge techniques. — Sedimentation coefficients, s, were measured as described previously[11] and recorded in Svedberg units, $S = 10^{-13}$ sec. The normalised weight-frequency functions, $g(s_o)$, for the limiting sedimentation-coefficients, s_o, were also determined, where

$$g(s_o) = \frac{1}{c_o}\frac{\mathrm{d}c_o}{\mathrm{d}s_o},$$

c_o being the total concentration[19]. The value of $g(s_o)$ was determined by monitoring the broadening with time of the boundary gradients formed in sedimentation-velocity experiments[19]. Such broadening is due to both diffusion and solute heterogeneity. To find $g(s_o)$, it was first assumed that diffusion is negligible. Further assumptions were that s is independent of concentration, and that the solvent and solute are incompressible. The distribution of sedimentation coefficients is then given by:

$$g(s_o) = \frac{1}{c_o}\frac{r^2}{r_a}\omega^2 rt\frac{\mathrm{d}c}{\mathrm{d}r},$$

where c is the concentration, r the radial distance to the boundary, r_a the radial distance to the meniscus, ω the angular velocity, and t the time[19]. Corrections are then made for the effects of diffusion, concentration, and pressure[19]. Clearly, the assumptions underlying this method of determining $g(s_o)$ are to some degree unjustified. However, the procedure does provide an estimate of $g(s_o)$ for each starch polymer-system and permits comparison of different samples. The observed distributions were checked to ensure that each gave the appropriate weight-average sedimentation coefficient.

Partial specific volume. — Partial specific volumes were determined with an AP Paar Precision System DMA60/602 Density Meter. Data were analysed by the procedure of Kratky *et al.*[20].

RESULTS AND DISCUSSION

The sedimentation coefficient and M_w *of amylopectin.* — The sedimentation coefficient in Me_2SO solutions of amylopectin isolated from *Aotea* wheat starch was found to vary linearly with, and depend markedly on, the concentration. This is consistent with previous work[11,21]. Extrapolation showed s_0 to be 103 ±16 S. Banks *et al.* reported[21] 280 S for the sedimentation coefficient of wheat starch amylopectin in water. Before these s_0 values can be compared directly, the sedimentation coefficient in Me_2SO must be corrected to take account of differences in the viscosities and densities of Me_2SO and water, and also differences in the partial specific volumes of the polymer in these solvents[19]. The partial specific volume of amylopectin in Me_2SO was found to be 0.59 $cm^3\ g^{-1}$ at 25° whereas the corresponding value for amylopectin in water is[21] 0.62 $cm^3\ g^{-1}$. Taking the viscosities of Me_2SO and water as 1.98 and 1.00 centipoises, respectively, gives 230 S for the sedimentation coefficient of amylopectin in Me_2SO that corresponds to the figure in water. Provided that the sedimentation coefficient does not vary markedly from one source of wheat starch amylopectin to another, as is shown here to be the case, then the difference between the sedimentation coefficient in water and the corrected value in Me_2SO suggests that the size and/or the shape of the polymer is different in these solvents.

The Svedberg equation relates s_0 to the molar mass M as follows:

$$M = \frac{s_0 RT}{D_0(1 - \nu\rho)} \qquad (1),$$

where R is the gas constant, T the absolute temperature, ν the partial specific volume, and ρ the density. When a range of polymer sizes is present, it is the weight-average diffusion coefficient, D_0, that is of concern. For amylopectin in Me_2SO, this has a value of $9 \times 10^{-12}\ M^2\ s^{-1}$ according to p.f.g.–n.m.r.[13]. This figure can be shown to agree with the z-average diffusion coefficient found by quasi-elastic light scattering[11,13]. Substitution in the Svedberg equation gives $\sim 10^7$ for M_w, which is in broad agreement with previous[13] estimates of M_w for amylopectin in Me_2SO.

Literature values[13,21] of s_0 and D_0 for amylopectin in water may also be used to estimate M_w. This procedure yields an M_w value of $\sim 6 \times 10^7$, which agrees with the figure of 8×10^7 obtained by substituting sedimentation and viscosity data[3] in the appropriate expression derived by Flory and Mandelkern[22]. However, light-scattering measurements in aqueous solvents indicate[7,8] M_w to be ~4 × 10^8. The difference between the M_w measurements for amylopectin in Me_2SO and water provides further evidence that aggregation occurs in the aqueous system. The extent of this aggregation would be expected to depend on the concentration of polymer. As M_w measurements on aqueous systems have been made over different concentration ranges of polymer, perhaps this accounts for the fact that the values reported for M_w in aqueous solution vary by a factor of about 35.

The value of s_0 *of amylopectin in unfractionated starch.* — The sedimentation coefficient of the amylopectin in *Aotea* wheat starch was also measured by using solutions of unfractionated starch in Me_2SO. This procedure shows two distinct peaks[11]. The fraction of greater molar mass gave a sedimentation coefficient of 105 ±2 *S*. The iodine-binding capacity of a sample of this fraction obtained by centrifugation[11] was 0.3%, showing it to be amylopectin. The distributions of sedimentation coefficients of amylopectin in Me_2SO obtained from a mixture of unfractionated starch and from a sample fractionated by the classical thymol procedure were found to be very similar. This finding, together with the fact that there is no significant difference in the s_0 values, suggests that classical fractionation is an effective means of obtaining a representative sample of amylopectin. It should be recognised, however, that this conclusion is based on the assumption that the Johnson–Ogston effect[19] which is present when unfractionated samples are sedimented, is negligible.

The value of s_0 *for starches from different wheat cultivars grown under various conditions.* — Table I shows the s_0 values for amylose and amylopectin from starches isolated from various wheat cultivars that had been grown under a number of conditions. The s_0 values reported previously for starch polymers in Me_2SO that had been obtained from a single wheat variety *(Flanders)* grown in Europe, lie in the range of s_0 values reported here. The distributions of sedimentation coefficients corresponding to the s_0 results for amylopectin shown in Table I were found to be similar to those shown in Fig. 1. As expected, the distributions shift slightly in

TABLE I

VALUES OF s_0 FOR AMYLOSE AND AMYLOPECTIN FROM STARCH ISOLATED FROM A NUMBER OF WHEAT CULTIVARS GROWN INVARIOUS LOCATIONS

Variety	*Growth location*	s_0 *(Svedberg units)*	
		Amylose	*Amylopectin*
Rongotea	Swanannoa Research Station	5.0 ±0.2	88 ±3
Rongotea	Oamaru Research Station	5.2 ±0.5	115 ±4
Rongotea	Courtney Research Station	4.5 ±0.1	103 ±1
Crossbow	Swanannoa Research Station	2.6 ±0.4	65 ±7
Crossbow	Oamaru Research Station	3.2 ±0.1	67 ±1
Crossbow	Courtney Research Station	3.3 ±0.1	73 ±6
Aotea	N.Z.	4.0 ±0.7	105 ±2
Karamu	N.Z.	5.7 ±0.9	87 ±8
Hilgendorf	N.Z.	2.9 ±0.6	98 ±4

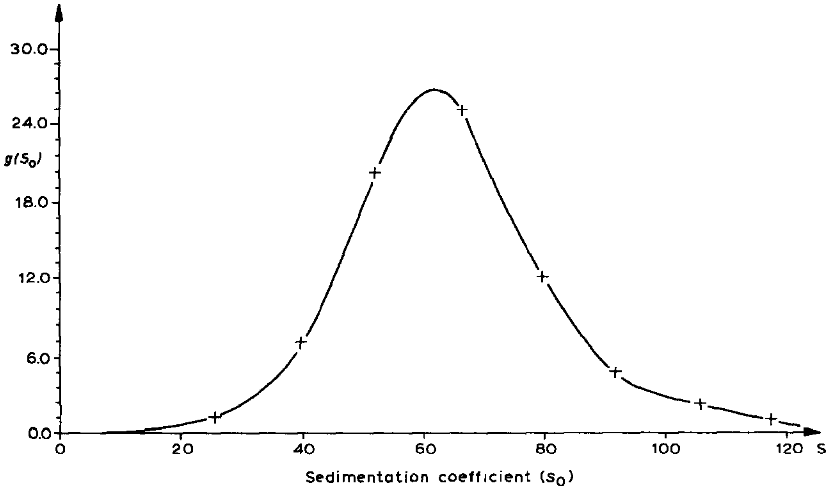

Fig. 1. Differential distribution of sedimentation coefficient (s_0) for amylopectin from *Crossbow* starch obtained from wheat grown at Swanannoa Research Centre.

accordance with changes in the s_0 value. Both the s_0 and $g(s_0)$ results reported herein suggest that there is some slight variation in the molecular size of wheat-starch amylopectin from one cultivar to another and from one growth location to another. However, the variation appears to be much less than in waxy maize amylopectin, where a five-fold difference between the sedimentation coefficients of two varieties has been reported.

Relation of s_0 *values and rheological properties.* — The s_0 and sedimentation-coefficient distributions for polymers from different cultivars and growth locations do not show any systematic variation with the rheological properties of the corresponding starch pastes[14]. This suggests that differences in the packing of the macromolecules into granules may be responsible for the range in properties found in wheat starches from different cultivars[23]. Variations in the trace amounts of lipid and protein occurring in granules may also contribute to differences in starch rheological behaviour.

Distributions of sedimentation coefficients. — Fig. 1 shows the distribution of sedimentation coefficients for the amylopectin peak of *Crossbow* starch obtained from wheat grown at Swanannoa Research Centre. The data have been corrected for diffusion and concentration effects[19]. Pressure effects were found to be negligible. The peak-width half-height is ~60 *S*. Relatively few distributions of sedimentation coefficients for biopolymers have been published. Geddes has reported such data for glycogen[24]. The distribution for amylopectin reported herein is unimodal and symmetrical, whereas Geddes found a bimodal distribution for glycogen in Me_2SO. The range of sedimentation coefficients for amylopectin is much less than that reported for glycogen in this solvent. However the experimental technique used in the present study would not be capable of measuring the very high sedimentation coefficients (of the order of 100 S) recorded by Geddes for glycogen. The low sedimentation coefficients detected in glycogen are not present in amylopectin, but do occur with amylose. Although the distributions of sedimentation coefficients established in the present study are much narrower than for glycogen, they are consistent with a wide range of molecular sizes[19].

Sedimentation coefficients and hydrodynamic theory. — Provided that M_w and v are known, sedimentation-coefficient data may be used to estimate the size and shape of polymer molecules[25]. The method assumes an ellipsoidal model for the polymer; an oblate ellipsoid is appropriate for amylopectin[13]. The frictional coefficient of the amylopectin is of concern. For spheres, the frictional coefficient, f_o, is defined by the Stokes equation:

$$f_o = 6\pi\eta N r \qquad (2),$$

where η is the viscosity of the solvent, N is Avogado's number, and r the radius of the sphere. For an oblate ellipsoid, the frictional coefficient, f, is expressed in terms of the ratio f_o/f, where f_o is for a sphere of the same volume as the ellipse.

$$f_o/f = F = \frac{J^{2/3}\tan^{-1}\sqrt{J^2-1}}{\sqrt{J^2-1}} \qquad (3).$$

F is a general structural parameter and $J = a/b$ is the ratio of the semi-axes of the particles. The volume of the oblate ellipsoid and its equivalent sphere are related as follows:

$$\frac{4}{3}\pi r^3 = \frac{4}{3}\pi a^2 b \qquad (4),$$

However, for spheres,

$$\mathrm{M} = \frac{4}{3}\pi r^3 \frac{N}{v} \qquad (5),$$

and hence

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

The frictional coefficient is also related to the diffusion coefficient; this is the Einstein relationship:

$$D_o = \frac{RT}{f} = \frac{RT}{f_o} F \qquad (7).$$

As s_0 and D_0 are measured in solutions of identical composition, the same frictional coefficient applies in each case. Hence, on combining Eqs. *1*, *6*, and *7*:

$$M^2 = \frac{162\,\pi^2\eta^3N^2s_0{}^3\nu}{F^3(1-\nu\rho)^3} \tag{8}$$

or

$$F = \left[\frac{162\,\pi^2\eta^3s_0{}^3\nu N^2}{M^2(1-\nu\rho)^3}\right]^{1/3} \tag{9}$$

Eq. *9* permits the size and assumed shape of amylopectin to be calculated. Substituting the values of M_w equal to $\sim 10^7$ and s_0 equal to 103 S gives $F = 0.460 \pm 0.004$.

Substituting this value into Eq. *3* gives the axial ratio J for the proposed oblate ellipsoid as ~38. This indicates that amylopectin molecules in Me_2SO have a flat sheet-like structure. Furthermore, by use of Eqs. *4* and *5*, we find that the molecular semi-axes are given by:

$$a = \left(\frac{3MJ\nu}{4\pi N}\right)^{1/3} \cong 45\,\text{nm} \qquad \text{and}\; b \cong 1.2\,\text{nm}.$$

In addition, the mean Stokes-radius R_B is of the order of 13 nm. It should be recognised that the experimental data upon which the analysis is made is weight averaged, and thus it is expected that the larger particles dominate.

While the previous n.m.r. study[13] and the present investigation both indicate that amylopectin is a highly planar molecule, there remains some uncertainty as to its precise dimensions. Nevertheless the theoretical models upon which the separate analyses are based are different, and so such a numerical discrepancy is not unexpected.

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