Static and Dynamic Light Scattering Studies of Amylose Solutions

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ABSTRACT: Solutions of amylose have been prepared by aqueous leaching from pea starch granules. Samples leached between 62 and 80 °C have been shown to be linear. Molecular weight fractions were prepared by selective leaching over controlled temperature intervals. For such fractions the dependence of the radius of gyration, the translational diffusion coefficient, and the intrinsic viscosity on molecular weight confirms the belief that amylose behaves as a non-free draining coil. The characteristic ratios and the persistence length were 11.7 and 27.8 Å, respectively. Calculated unperturbed values of the characteristic ratio and persistence length are ≈4.5 and 12.1 Å, respectively. Calculations of molecular size from static and dynamic light scattering are dependent on the breadth of the molecular weight distribution. It has been demonstrated how this dependence may be used to fit simple two-parameter molecular weight distribution functions.

Introduction

Starch, one of the most abundant of natural biopolymers, is the major reserve polysaccharide of higher plants where it occurs in the form of water insoluble granules. The polysaccharides amylose and amylopectin may be extracted from the granule. Most starches contain ≈25% amylose and ≈75% amylopectin.[1] Both polymers contain chains of (1→4)-linked glucosyl residues.[1] Amylose is essentially linear whereas amylopectin is highly branched.[1] Branches are linked at the 6-position.[1]

Upon heating an aqueous dispersion of starch granules at, or above, a characteristic temperature, known as the gelatinization temperature, an irreversible transition is observed.[1,2] The granules swell to many times their original size and amylose is preferentially leached into solution. Cooling such a solution results in a phenomenon termed retrogradation.[2] This is believed to involve aggregation and subsequent crystallization of the amylose fraction of the sample.[1-3] The result is opaque viscoelastic pastes or, at higher starch concentrations, opaque elastic gels. Retrogradation of starch and amylose preparations is an area of considerable commercial importance in the food industry.

Basic studies of retrogradation require the preparation of well-characterized molecular solutions of amylose. The preparation of true solutions of polysaccharides is notoriously difficult[4] and light scattering studies on amylose preparations[5,6] suggest that these samples are often contaminated with amylopectin, retrograded amylose or impurities such as dust. Light scattering may be used to assess the "cleanliness" of samples. For true solutions it is possible to determine the molecular size, shape, and rigidity. Published light scattering data[1,7-10] on amylose solutions suggest that the polymer behaves as a non-free draining coil. However, there is a wide variation in the reported stiffness of the polymer chain. Investigations on neutral (0.33 M KCl) solutions of potato amylose[1] yield characteristic ratios (C_∞) ≈5–6 whereas studies on aqueous solutions of synthetically produced amylose fractions[9] suggest $C_\infty \approx 11$–12. Light scattering studies[7-10] of amylose fractions in dimethyl sulfoxide (Me_2SO) suggest $C_\infty > 10$. The value of $C_\infty \approx 5$–6 is employed in computer simulations of molecular shape and dynamics.[6,11]

In the present studies care has been taken to select preparative procedures which eliminate the presence of amylopectin, retrograded amylose, and extraneous impurities. Static and dynamic light scattering methods have been employed to measure molecular size, shape, and stiffness. Present data have been compared with previous light scattering studies. Branching may strongly affect molecular size and shape. Recent enzymatic studies[12] suggest that potato amylose may be more highly branched than previously suspected. Since the majority of light scattering studies have been concerned with potato amylose, the present studies have been carried out on pea amylose, an inherently more linear form of amylose.[13] In addition, detailed enzymatic procedures have been employed to check for possible branching.

Materials and Methods

Starch was extracted from smooth-seeded peas (Filby) by the aqueous extraction method of Adkins and Greenwood.[14] The purified starch had an amylose content of 25% as assessed by its iodine binding capacity determined by using a semi-micro differential potentiometric method.[15] The gelatinization temperature of the starch, defined as the temperature at which in aqueous dispersion the granules irreversibly swell to many times their original size, was found to be 62 °C. Amylose fractions of various molecular weights were obtained by successive aqueous leaching of the granules in intervals of 5 °C over the temperature range 62–85 °C. Swollen granules were removed by mild centrifugation (~2000*g*) and filtration through a glass sinter (porosity 2). Samples were allowed to cool to room temperature, filtered through 0.22-μm cellulose acetate filters, and diluted with filtered solvent to the required concentration. Light scattering studies were made within 2–3 h of preparation. No systematic time-dependent changes in scattered intensity were observed during these studies. However, over periods of >12 h extensive precipitation of amylose was observed. The absence of any sharp curvature of the angular scattering plots at low angles was taken to imply that the solutions were free of aggregates or "large" impurities. A further check involved studying the effect on the scattering envelope of the addition of the enzyme α-amylase (*B. subtilis*, Sigma) to the amylose solutions. The following procedure was employed: A 0.1% w/w aqueous solution of the enzyme was prepared. This contained 50–100 units of enzyme activity. One unit will liberate 1 mg of maltose from starch in 3 min. The enzyme solution was clarified by filtration (0.22 μm) and 20 μL carefully added to the amylose solution (typically 10 mL at a concentration of ~1 mg/mL). The intensity of the scattered light was found to decrease rapidly and become constant within about 1 h. This constant value was found to be virtually indistinguishable from the solvent scattering (Figure 1). Both checks were consistent with a true molecular solution.

Preparative techniques involving precipitation and subsequent redispersion[16] were avoided because preliminary studies showed that it was difficult to prepare aggregate-free solutions using such methods.

Polymer concentrations were measured by using the phenol–sulfuric acid colorimetric method.[17] Both chemical and enzymatic methods were used to check the purity of the amylose preparations. The iodine binding capacity was 19.5 ± 0.5 mg of iodine per 100 mg of polysaccharide under standard conditions. This suggests highly pure amylose preparations containing less than ~2–3% of amylopectin. Linearity of the amylose molecules was

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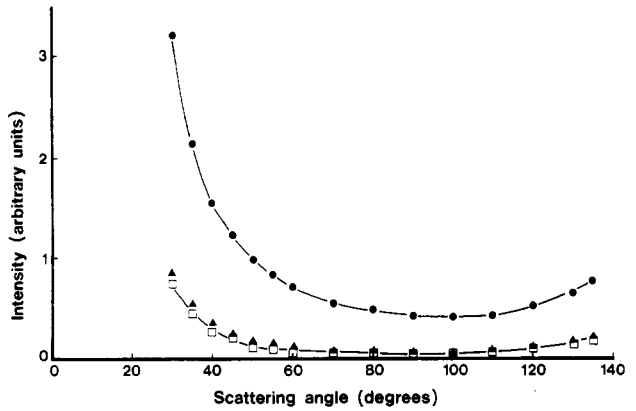

Figure 1. Effect of enzymatic digestion on the polar scattering diagram for 0.15 mg/mL amylose solution: (●) scattering from solution; (▲) scattering from solution after enzyme digestion for 4 h; (□) solvent scattering.

assessed by monitoring the degradation of the amylose to maltose using a β-amylase (sweet potato, Sigma). Samples prepared by aqueous leaching in the temperature range 60–80 °C showed 100% conversion to maltose, indicating no detectable branching. Fractions leached between 80 and 85 °C showed 90% conversion implying some degree of branching. It could be argued that these results implied the presence of amylopectin rather than branched amylose. Amylopectin has a β-amylolysis limit of ~50%. The level of contamination of an amylose sample with amylopectin necessary to produce an average β-amylolysis limit of 90% would be 20% contamination. This is clearly inconsistent with the iodine binding capacity of this fraction. Although the possibility of contamination with trace amounts (~2–3%) of amylopectin cannot be completely ruled out, it was concluded that the 80–85 °C fraction contained a substantial amount of branched amylose.

When β-amylase is used to assess linearity it is essential to check the purity of the enzyme preparations. Contamination with α-amylase or α-glucosidase will lead to misleading results. β-Amylase is an exoacting enzyme which specifically cleaves maltose from the nonreducing end of an amylosic chain.[18] The action of the enzyme is hindered by the presence of derivatized glucosyl residues and hence the degree of degradation monitors the extent of branching of the amylose molecule. Contamination of β-amylase preparations with the endoacting enzyme α-amylase permits the bypassing of such obstructions and hence a high degree of subsequent degradation by β-amylase. A pure β-amylase preparation will not release derivatized maltose residues from a colored amylose derivative such as amylose azure.[18] If the preparation is contaminated with α-amylase, then the polymer is degraded and low molecular weight oligomers containing the chromophores are produced. The commercial β-amylase (20 units) was added to 0.1% w/w amylose azure in 0.02 acetate buffer at pH 5 in dialysis tubing and kept at ~30 °C for 24 h. No release of azure color into the surrounding medium was detected. Deliberate contamination of the β-amylase preparation with α-amylase resulted in detectable release of color. Contamination of β-amylase preparations with α-glucosidase will also lead to anomalously high degradation values. The degradation of amylose to maltose is conveniently detected by assaying for reducing end groups. α-Glucosidase hydrolyses maltose to glucose, thus yielding double the expected number of reducing end groups.[18] However, the reducing power of a maltose solution did not increase when incubated with the commercial β-amylase preparation, inferring the absence of α-glucosidase.

Thus β-amylase digestions were carried out in the following manner: 20 units of β-amylase was incubated with 10 mL of 0.1% w/w amylose in 0.02 M acetate buffer at pH 5 for 4 h at 30 °C. The relatively high enzyme level was employed in order to ensure rapid β-amylolysis and thus to minimize the chance of retrogradation of low molecular weight oligomers occurring during the experiment. The reducing sugar value was determined by the method of Nelson and Somogyi.[19]

Preliminary static light scattering studies were made by using an Aminco light scattering photometer belonging to Dr. D. B. Sellen (Astbury Department of Biophysics, University of Leeds, U.K.). The incident light was unpolarized and the wavelength was 546 nm. The majority of light scattering studies were made by using a modified Malvern 4300 spectrometer system. The incident light was an unfocused vertically polarized beam of wavelength 442 nm. Glass scintillation vials (diameter 2.5 cm) were used as scattering cells. Alignment of the instrument was checked by measuring the angular dependence of (i) light scattered by filtered benzene samples and (ii) fluorescence of dilute fluorescein solutions. Satisfactory alignment was achieved in the angular range 30–150°. Measurements of the Rayleigh ratio were made relative to benzene, and the Rayleigh ratio of benzene was taken[20] to be $R_{90°} = 64.23 \times 10^{-6}$ m^{-1}. Depolarization effects for amylose solutions were assumed to be negligible. Scattered intensities were averaged over periods of 0.5 s and monitored for 25 s. Results were recorded over the angular range 30–135°. The refractive index increment at 442 nm was taken to be 0.15 mL g^{-1} for aqueous amylose solutions.[21] Absolute calibration of the photometer and techniques for clarifying solutions were assessed by measuring the molecular weight of bovine serum albumin (Sigma). At 24 °C in 0.01 M NaCl at a pH of 6.8 a value of $72.9 \pm 7) \times 10^3$ was found in accord with literature values.[22] Data on amylose solutions were analyzed by using the Zimm plot procedure.[23] Zimm plots occasionally showed evidence of slight downward curvature at the lowest scattering angle, 30°. The effect only occurred in very dilute solutions and was only apparent when the excess scattering due to the amylose in solution was computed by subtracting the solvent scattering from the solution scattering. If the polymer was digested with α-amylase and the scattering from this digest used as a solvent scattering background then the curvature at 30° disappeared. It is possible that this indicated the presence of small quantities of retrograded amylose. However, the asymmetry of the scattering from the α-amylase "digest" at low angles is only slightly greater than the asymmetry of the solvent scattering. Thus it has been assumed that the effect arises due to small quantities of dust contaminating the solutions. α-Amylase digestion was used to justify the value of the preparative technique but was not used routinely for evaluating "background" scattering. Thus, any slight downward curvature of Zimm plots below 40° was attributed to residual dust and those data points omitted in evaluating radii of gyration and molecular weight.

Quasi-elastic light scattering studies were made by using single-clipped homodyne detection[24] at a wavelength of 442 nm using a standard Malvern 4300 spectrometer system. Measurements were made over the angular range 30–135° at a temperature of 24 °C. Autocorrelation functions could be adequately described by weighted second-order polynomials. The calibration of the instrument and the preparative procedures were checked by studies on bovine serum albumin solutions. In 0.01 M NaCl, pH 6.8 at 24 °C, the translational diffusion coefficient $\langle D_T \rangle_z$ was found to be $(5.6 \pm 0.1) \times 10^{-11}$ m^2 s^{-1}, in satisfactory agreement with published data.[24]

Viscosity measurements were made at 25 °C by using standard Ostwald capillary viscometers. The use of capillary viscometers to characterize dilute solutions of amylose has been discussed by Banks and Greenwood.[1] Data were collected on filtered amylose solutions in the concentration range 0.2–2 mg/mL. In this concentration range the reduced viscosity was found to be independent of concentration. Values of intrinsic viscosity $[\eta]$ were obtained after extrapolation of experimental data to zero concentration.

Results

Static light scattering results obtained by using both the Aminco and the Malvern spectrometer systems agreed within the limits of experimental accuracy. Figure 1 shows the effect of α-amylase digestion on the angular dependence of the scattered light. Typical Zimm diagrams for a low and high molecular weight fraction are shown in Figures 2 and 3. Typical values of the z-average square of the radius of gyration $\langle R_g^2 \rangle_z$ and the weight-average molecular weight $\langle M \rangle_w$ obtained for fractions leached over different temperature ranges are given in Table I.

Figure 4 shows a typical second-order autocorrelation function and the theoretically fitted second-order poly-

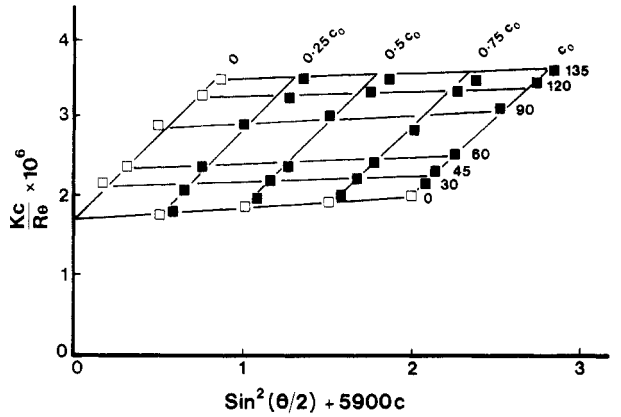

Figure 2. Zimm plot obtained for a 65–70 °C fraction at 24 °C: wavelength, 442 nm; c_0 = 0.34 mg/mL.

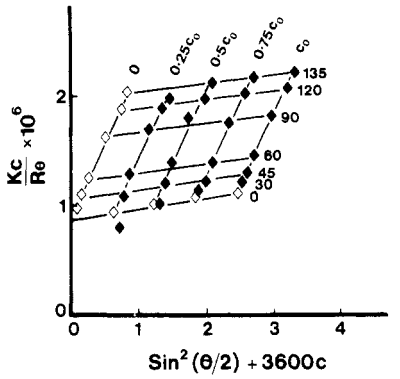

Figure 3. Zimm plot obtained for a 80–85 °C fraction at 24 °C: wavelength, 442 nm; c_0 = 0.68 mg/mL.

Table I

leaching temp, °C	$\langle M\rangle_w \times 10^{-6}$, daltons	$\langle D_T\rangle_z \times 10^{12}$, m² s⁻¹	$\langle R_g^2\rangle_z \times 10^{16}$, m²	$(R_g^2)_{dynamic}{}^a \times 10^{16}$, m²
62–65	0.27	13.1	9.2	7.5
65–70	0.57	10.5	25.4	15.6
70–75	0.84	10.1	31.0	17.8
75–80	0.90	9.0	31.9	20.2
80–85	1.11	8.4	33.5	22.2

[a] Values of $(R_g^2)_{dynamic}$ have been computed from eq 4 assuming ξ = 0.665.

nomial. Figure 5 shows the dependence of the initial slope of the autocorrelation function $\langle\Gamma\rangle_z = 2q^2\langle D_T\rangle_z$, estimated from the first coefficient of the polynomial fit, on the square of the modulus of the scattering vector $q^2 = (4\pi n_0/\lambda)\sin^2(\theta/2)$. λ is the vacuum wavelength of the incident light, n_0 the refractive index of the solvent, and θ the scattering angle. Thus values of $\langle D_T\rangle_z$ were found to be independent of scattering angle and no significant concentration dependence was observed in the concentration range 0.2–2 mg/mL. Values of $\langle D_T\rangle_z$ are listed in Table I. The absence of tails in the correlation function provides further evidence for the absence of amylopectin or retrograded amylose in the samples studied.

Discussion

Amylose fractions used in the present study were prepared by selective aqueous leaching from the starch granule. The enzyme digestion studies (Figure 1) and the angular dependence of the scattered light (Figures 2 and 3) demonstrate the absence of amylopectin or aggregates. The iodine binding studies provide further evidence for the purity of the samples and the absence of amylopectin. Enzymatic studies show that aqueous leaching in the

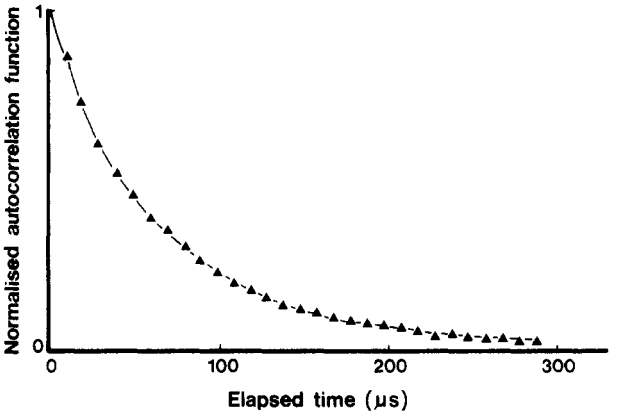

Figure 4. Typical normalized second-order correlation function obtained for a 1.3 mg/mL amylose (62–65 °C fraction) solution. The incident wavelength is 442 nm, the scattering angle 90°, and the temperature 24 °C: (▲) experimental data; (—) theoretical weighted second-order polynomial fit.

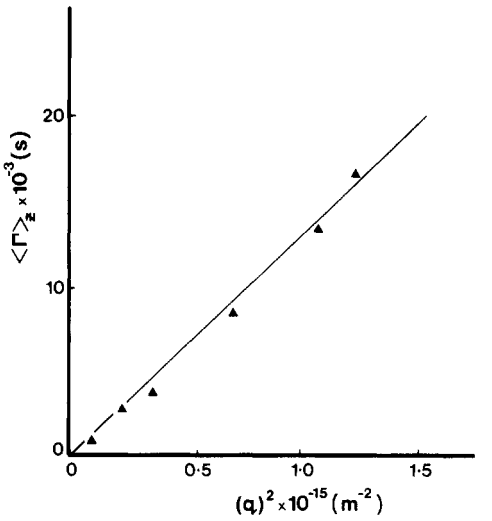

Figure 5. Dependence of the initial slope of the correlation function $\langle\Gamma\rangle$ on the square of the modulus of the scattering vector (q^2). Amylose fraction (62–65 °C): concentration 1.3 mg/mL; wavelength, 442 nm; temperature, 24 °C.

temperature interval 60–80 °C yields linear molecules. Leaching above 80 °C leads to some degree of branching. The data in Table I demonstrate that as the leaching temperature is raised, amylose fractions of increasing molecular mass and size are solubilized.

Molecular size and shape may be determined by analyzing the dependence of $\langle D_T\rangle_z^{-2}$, $[\eta]$, and $\langle R_g^2\rangle_z$ on molecular weight $\langle M\rangle_w$. The nature of the average value of $[\eta]$ is ill-defined.

$$\langle R_g^2\rangle_z = K_\alpha\langle M\rangle_w{}^\alpha \quad (1)$$

$$[\eta] = K_\beta\langle M\rangle_w{}^\beta \quad (2)$$

$$\langle D_T\rangle_z{}^{-2} = K_\gamma\langle M\rangle_w{}^\gamma \quad (3)$$

The molecular weight range available for investigation is limited by two factors. The lower limit of molecular weight is dictated by the gelatinization temperature for pea starch. In principle, lower molecular weight fractions could be obtained by hydrolysis of the lowest molecular fractions obtained by leaching. However, aqueous amylose preparations with molecular weights less than 1×10^5 are extremely difficult to study due to rapid retrogradation. The upper limit to the molecular weight range is determined by the appearance of branching of the amylose molecule. Branching will affect the overall dimensions of the mole-

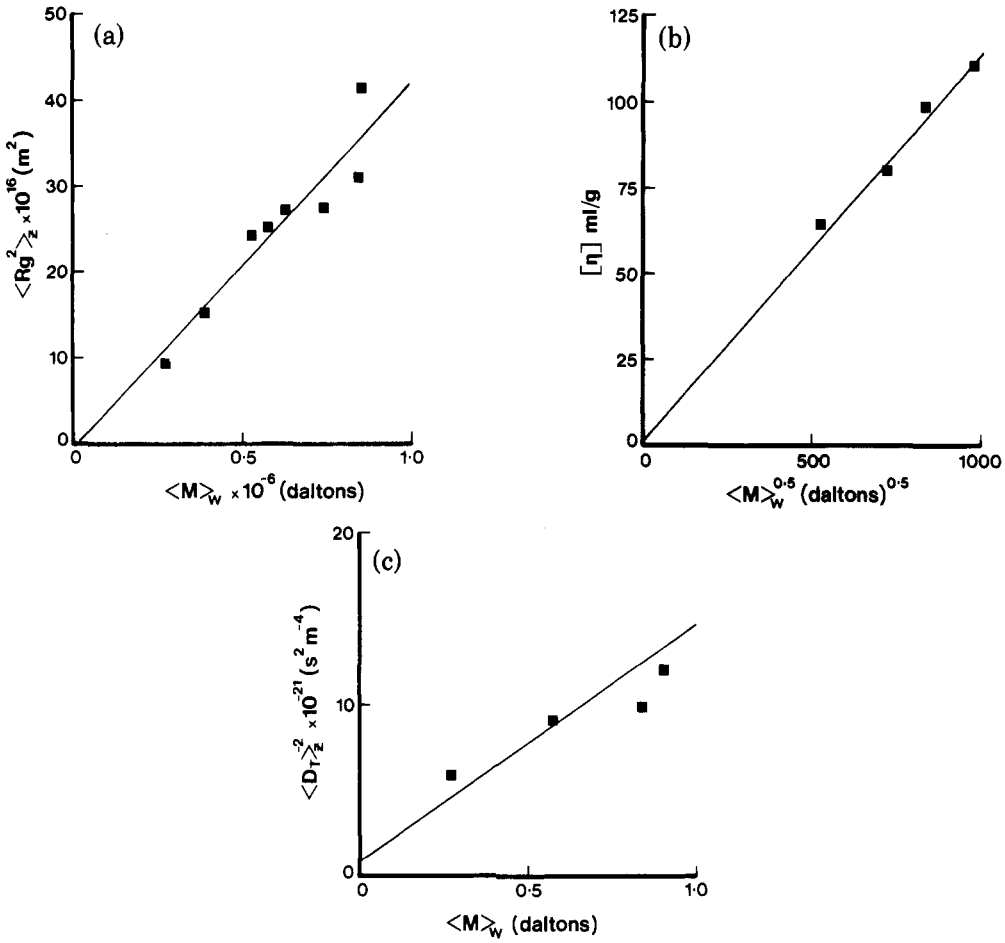

Figure 6. Molecular weight dependence of the parameters $\langle R_g^2\rangle_z$, $[\eta]$, and $\langle D_T\rangle_z^{-2}$ for linear amylose fractions. Data obtained for branched amylose fractions have been omitted. The full lines are least-squares fits as tabulated in Table II: (a) $\langle R_g^2\rangle_z$ from static light scattering; (b) $[\eta]$ intrinsic viscosity; (c) $\langle D_T\rangle_z^{-2}$ from dynamic light scattering.

Table II

parameter,[a] P	i	K_i	A_i	correl coeff
$[\eta]$ (mL/g)	0.5	0.113 (mL/(g dalton))	1.31 (mL/g)	0.997
$\langle R_g^2\rangle_z$ (m^2)	1.0	4.3×10^{-21} (m^2/dalton)	-0.61×10^{-16} (m^2)	0.97
$\langle D_T\rangle^{-2}$ (s^2/m^4)	1.0	1.67×10^{22} ($s^2/(m^4$ dalton))	9.4×10^{20} (s^2/m^4)	0.985

[a] The dependences of the parameters $[\eta]$, $\langle D_T\rangle_z^{-2}$, and $\langle R_g^2\rangle_z$ on molecular weight were assessed by least-squares fitting of the data to a straight line $P = K_iM^i + A_i$. The variable $i = \alpha$, β, and γ for P equal to $\langle R_g^2\rangle_z$, $[\eta]$, and $\langle D_T\rangle_z^{-2}$ respectively is defined in eq 1-3.

cule in solution and hence R_g, $[\eta]$, and D_T.

Experimental plots showing the variations of $\langle R_g^2\rangle_z$ (from static light scattering), $[\eta]$, and $\langle D_T\rangle_z$ are shown in Figure 6. Results of the analysis of these data are shown in Table II. The parameters α, β, and γ support previously published data[1,7-10] and also the conclusion that in dilute, neutral aqueous solution amylose behaves as a non-free draining coil.[1]

For monodisperse non-free draining coils[22]

$$D_T = \frac{kT}{6\pi\eta\xi R_g} \tag{4}$$

where k is the Boltzmann constant, T the absolute temperature, η the viscosity of the solvent, and ξ a proportionality constant relating the radius of gyration to the hydrodynamic radius ($R_h = \xi R_g$). The theoretically calculated value[22] of ξ is 0.665. Recent attempts to determine ξ experimentally have questioned assumptions used in the derivation of ξ.[25,26] Until this matter is resolved it will be assumed that the theoretical value of $\xi = 0.665$ is correct and interpretation of results based on this assumption. Values of R_g^2 calculated by assuming[22] $\xi = 0.665$ are compared with values found from static light scattering studies in Table I. For coil-like molecules the difference in R_g^2 values obtained by static and dynamic light scattering will be dependent on the value of ξ and the breadth of the molecular weight distribution. If the choice of ξ is correct, then the differences result solely from polydispersity. The experimentally measured average quantities will be of the form

$$\langle D_T\rangle_z = \frac{\sum_i N_iM_i^2D_{Ti}}{\sum_i N_iM_i^2} \tag{5}$$

$$\langle R_g^2\rangle_z = \frac{\sum_i N_iM_i^2R_{gi}^2}{\sum_i N_iM_i^2} \tag{6}$$

$$\langle M\rangle_w = \frac{\sum_i N_iM_i^2}{\sum_i N_iM_i} \tag{7}$$

For non-free draining coils[22]

$$R_g^2 = \frac{n\alpha^2l_e^2}{6m} \qquad M = M_s\frac{n}{m} \tag{8}$$

where M_s is the mass and l_e the length of the Kuhn statistical segment, m the number of structural units per molecule, (n/m) the number of statistical segments, and

Table III

leaching temp,[a] °C	$M_0 \times 10^{-6}$, daltons	σ	$\langle M\rangle_w/\langle M\rangle_n$
62–65	0.21	0.52	1.31
65–70	0.30	0.81	1.93
70–75	0.40	0.86	2.10
75–80	0.49	0.78	1.84

[a] Data obtained over the temperature interval 80–85 °C have not been analyzed because enzymatic studies indicate detectable branching. Values of $\langle M\rangle_w/\langle M\rangle_n$ have been calculated by using the relation $\langle M\rangle_w = \langle M\rangle_n \exp(\sigma^2)$.

α a parameter which is sensitive to segment–segment and segment–solvent interactions.[22] If the summations over species i in eq 5–7 are replaced by integrals then

$$\langle D_T\rangle_z = \frac{kT}{6\pi\eta\xi}\left(\frac{6M_s}{\alpha^2 l_e^2}\right)^{1/2}\frac{I(3/2)}{I(2)} \quad (9)$$

$$\langle R_g^2\rangle_z = \left(\frac{\alpha^2 l_e^2}{6M_s}\right)\frac{I(3)}{I(2)} \quad (10)$$

$$\langle M\rangle_w = \frac{I(2)}{I(1)} \quad (11)$$

where

$$I(j) = \int_0^\infty M^j f(M)\, dM \quad (12)$$

and $f(M)$ is the number distribution function of molecular weights. In eq 9–12 it has been assumed that the lower limit of integration may be taken to be zero. In practice, molecular shape would change if the molecular mass dropped below a characteristic threshold value M'. The effect of truncation of the integral (eq 12) has been discussed elsewhere.[27] Clearly provided

$$\int_0^{M'} M^j f(m)\, dM \ll \int_0^\infty M^j f(m)\, dM \quad (13)$$

then eq 9–12 will be valid. Thus for polydisperse samples

$$Q = \frac{\langle D_T\rangle_z^2\langle R_g^2\rangle_z}{(kT/6\pi\eta\xi)^2} = \frac{I^2(3/2)I(3)}{I^3(2)} > 1 \quad (14)$$

This accounts for the disagreement between R_g^2 values as determined by static and dynamic light scattering methods (Table I). The quantity Q specified in eq 14 will be determined by the choice of ξ and the breadth of the distribution function $f(M)$ and hence provides an index of polydispersity.

If the distribution function may be approximated by a simple two-parameter skew distribution function then 11, 12, and 14 suffice to evaluate $f(M)$. As an example consider the log–normal distribution[28]

$$f(M) = \frac{1}{\sigma M(2\pi)^{1/2}}\exp\left\{-\frac{1}{2}\left(\frac{\ln(M/M_0)}{\sigma}\right)^2\right\} \quad (15)$$

where M_0 is the geometric mean molecular weight and $\exp(\sigma)$ is the geometric standard deviation. Monodisperse sample have $\sigma = 0$. Then

$$I(j) = M_0^j \exp(j^2\sigma^2/2) \quad (16)$$

$$Q = \exp(3\sigma^2/4) \quad (17)$$

$$\langle M\rangle_w = M_0 \exp(3\sigma^2/2) \quad (18)$$

The analysis presented above is prefectly general. Provided ξ is known then eq 17 may be used to evaluate σ and then eq 18 may be used to evaluate M_0. The analysis of the data may be illustrated by assuming $\xi = 0.665$. Values of M_0 and σ calculated for the fractions leached over different temperature intervals are listed in Table III. The calculated molecular weight distributions are shown in Figure 7. An alternative method of specifying polydispersity is to quote the ratio of weight-average $\langle M\rangle_w$ and number-average $\langle M\rangle_n$ molecular weights ($\langle M\rangle_w/\langle M\rangle_n = \exp(\sigma^2)$. Values of this ratio are also listed in Table III. Should subsequent theoretical or experimental work modify the presently used value of ξ then the new value of ξ may be used in conjunction with eq 14, 17, and 18 to recalculate M_0 and σ.

The dependence of the radius of gyration on molecular weight may be used to estimate the stiffness of the macromolecules. The values of $\langle R_g^2\rangle_z$ determined by static light scattering are compared with data obtained by other authors in Figure 8. Only data relevant to neutral aqueous solutions are shown in Figure 9. The data due to

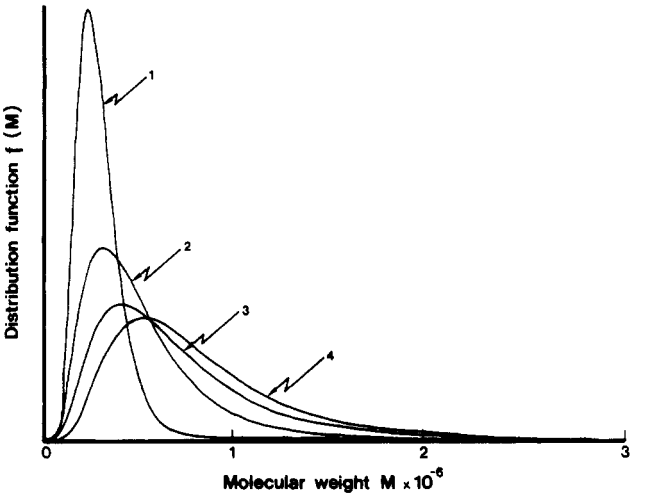

Figure 7. Calculated molecular weight distributions for amylose fractions. 1 (62–65 °C) fraction, 2 (65–70 °C) fraction, 3 (70–75 °C) fraction, 4 (75–80 °C) fraction.

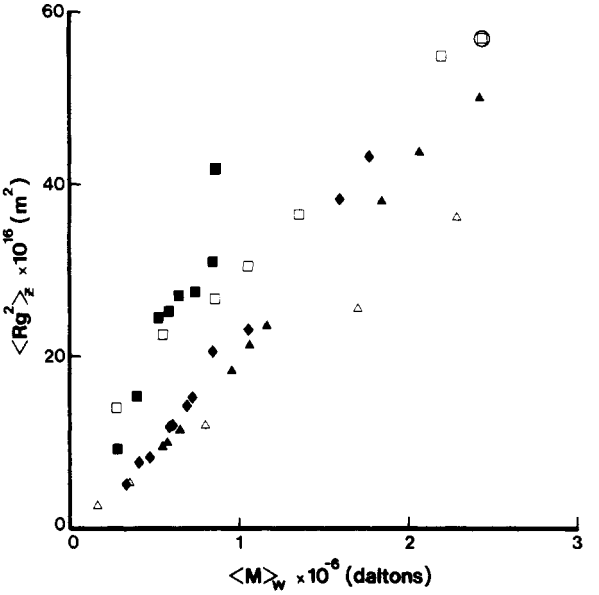

Figure 8. Plots of $\langle R_g^2\rangle_z$ vs. $\langle M\rangle_w$ for data obtained by various authors on dilute aqueous solutions of amylose: (■) present authors (solvent water); (♦) Burchard[9] (solvent water); Everett and Foster[10,29] (◎) solvent 0.5 N KCl; (□) corrected from Me_2SO to 0.5 N KCl as solvent); (△) Banks and Greenwood[1] (solvent 0.33 M KCl) and (▲) Kodama et al.[30] (solvent water).

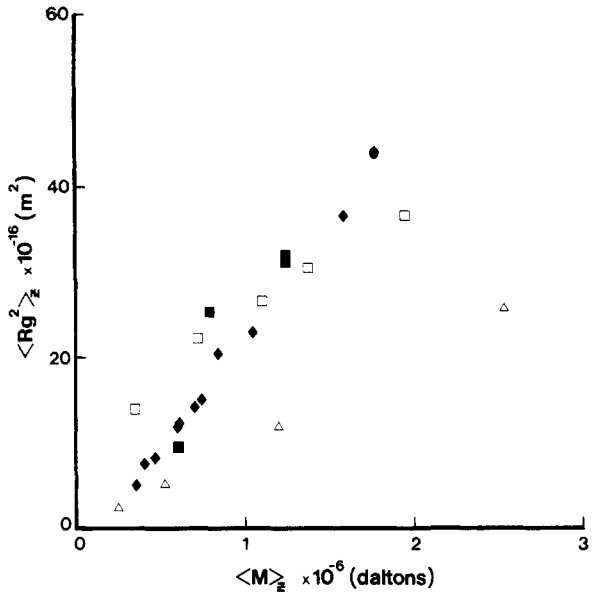

Figure 9. Plots of $\langle R_g^2 \rangle_z$ vs. $\langle M \rangle_z$ after correction for polydispersity (see text): (■) present authors; (♦) Burchard;[9] (□) Everett and Foster;[10,29] (△) Banks and Greenwood.[1]

Burchard[9] were obtained on synthetically produced amylose and the samples are considered to be monodisperse. The data due to Everett and Foster are a mixture of measurements made in 0.5 N KCl and data measured in Me_2SO as solvent and then corrected to 0.5 N KCl as a solvent. The method of correction for polymer expansion was suggested by Everett and Foster.[29] Viscosity measurements were made in both solvents and the ratio of polymer size between solvents calculated. This ratio was then used, for each molecular weight fraction, to convert the radii of gyration measured by light scattering. This approximation was checked by direct measurement of the radius of gyration in both solvents. It is clear from Figure 8 that the collected data are spread fairly broadly about the values obtained by Burchard.[9] A likely explanation for this spread of experimental data is polydispersity. Banks and Greenwood[1] suggest that polydispersity may be taken into account by converting $\langle M \rangle_w$ values to $\langle M \rangle_z$ values and comparing the $\langle R_g^2 \rangle_z$ with $\langle M \rangle_z$ rather than $\langle M \rangle_w$. Figure 9 shows an attempt to correct for polydispersity on the assumption of the distribution function $f(M)$ (eq 15). The data due to Banks and Greenwood have been corrected on the basis of their suggestion that $\langle M \rangle_w / \langle M \rangle_n = 1.5$. For a log-normal distribution $\langle M \rangle_w / \langle M \rangle_n = \langle M \rangle_z / \langle M \rangle_w$. Our results for linear fractions of amylose have been corrected on the basis that $\langle M \rangle_z = \langle M \rangle_w \exp(\sigma^2)$. The data due to Kodama et al.[30] contain no information on polydispersity and cannot be corrected. Everett and Foster[10] estimate $\langle M \rangle_z / \langle M \rangle_w = 1.3$. The data points due to Everett and Foster shown on Figure 9 have been obtained by assuming $\langle M \rangle_z / \langle M \rangle_w$ is constant for all fractions.

The corrections for polydispersity, approximate as they may be, improve the agreement between present data and that due to Burchard[9] and Everett and Foster.[10] However, there is marked disagreement with the data due to Banks and Greenwood.[1] The stiffness of the polymer chains may be monitored by calculating the characteristic ratio C_∞ defined[31] as

$$C_\infty = \frac{6\langle R_g^2 \rangle_w}{\langle n \rangle_w l^2} = \frac{6\langle R_g^2 \rangle_z}{\langle n \rangle_z l^2} \tag{19}$$

where l is the projected length of the "monomer" unit and $\langle n \rangle$ the appropriate average number of such units per molecule. Assuming no preferred secondary structure then $l = 4.4$ Å and $\langle M \rangle = 164\langle n \rangle$. Our data and that of Everett and Foster[10] agree with the data obtained by Burchard[9] on monodisperse amylose. These data suggest $C_\infty \approx 11.7$. Since[31]

$$C_\infty = \frac{2a}{l} - 1 \tag{20}$$

where a is the persistence length then $a = 27.8$ Å. This is substantially larger than the value obtained by Banks and Greenwood[1] ($C_\infty = 5.6$–5.9, $a = 14.5$–15.2 Å). Banks and Greenwood[1] obtained their data by using 0.33 M KCl as solvent and the reported second osmotic virial coefficient (A_2) was zero. The present data and that due to Burchard[9] have been measured by using water as a solvent and in both cases A_2 is positive. Positive A_2 values imply that α^2, as defined in eq 8, is greater than unity and thus that the measured radius of gyration ($\langle R_g^2 \rangle_z$) will be greater than the unperturbed value $\langle R_g^2 \rangle_0$. This expansion could account for the different C_∞ values. For a degree of polymerization (P) of 3650 Burchard[9] found $A_2 = 1.1 \times 10^{-4}$ cm^3 mol g^{-2} and $\alpha^2 = 2.46$. He reports $(R_g^2)_0 = 13.15P$ Å^2 corresponding to an unperturbed characteristic ratio of $C_\infty' = 4.1$ in good agreement with the data of Banks and Greenwood.[1] Values of α^2 may be calculated from the Flory–Orofino equation[32]

$$A_2 = \frac{16\pi}{(3)^{3/2}} \frac{N}{M^2} \langle R_g^2 \rangle_z^{3/2} \ln \left[1 + \frac{\pi^{1/2}}{2} (\alpha^2 - 1) \right] \tag{21}$$

where N is Avogadro's number. However, Burchard[9] has shown that for $M \approx (0.16 \times 10^6 - 0.65 \times 10^6)$ A_2 shows anomalous behavior and increases with increasing molecular weight. At higher molecular weights A_2 falls as expected with increasing molecular weight and α^2 may be estimated from eq 21. For the 65–70 °C fraction $\langle M \rangle_w = 0.84 \times 10^6$ and $A_2 = 4.8 \times 10^{-4}$ cm^3 mol g^{-2}. Putting $M = \langle M \rangle_z = \langle M \rangle_w \exp(\sigma^2)$, one obtains $\alpha^2 = 2.84$. Using eq 19, one obtains $C_\infty' = C_\infty/\alpha = 4.5$, again compatible with the data due to Burchard[9] and Banks and Greenwood.[1] Thus, the present data are compatible with that obtained by Burchard[9] in water and the calculated unperturbed dimensions are compatible with these measured by Banks and Greenwood[1] in 0.33 M KCl treated as a Θ solvent.

Conclusions

Present studies suggest that true molecular solutions of amylose may be prepared by aqueous leaching from pea starch granules. Leaching in the temperature range 62–85 °C yields samples free from detectable amylopectin. Samples leached in the range 62–80 °C are linear and show no detectable branching. True molecular solutions free from retrograded amylose may be prepared. The dependence of $\langle R_g^2 \rangle_z$, $\langle D_T \rangle_z^{-2}$, and $[\eta]$ on molecular weight are consistent with the belief that amylose behaves as a nonfree draining coil in dilute aqueous solution. Estimates of molecular size and stiffness are sensitive to the polydispersity of the samples. Comparison of measured $\langle R_g^2 \rangle_z$ and $\langle D_T \rangle_z$ values yields an index of polydispersity. A method has been suggested for evaluating molecular weight distributions and sample calculations performed. A major limitation of the method will be dictated by the experimental accuracy with which $\langle D_T \rangle_z$ and $\langle R_g^2 \rangle_z$ may be determined. These errors will set a lower limit to the width of the distribution which may be measured. However, studies on fractions leached over various temperature ranges are consistent with an increase in molecular size and mass with increased leaching temperature. The use of

static and dynamic light scattering to determine molecular weight distributions would benefit from the evaluation of ξ using monodisperse amylose preparations. Estimates of molecular stiffness are sensitive to polydispersity. Values corrected for polydispersity are consistent with $C_\infty = 11.7$ and $a = 27.8$ Å, in dilute aqueous solution. Corrections for chain expansion yield $C_\infty' \approx 4.5$ for the unperturbed state consistent with the data obtained in the Θ solvent 0.33 M KCl.

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^{13}C Relaxation of Dissolved Polymers Containing In-Chain Six-Membered Alicyclic Rings

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ABSTRACT: Spin-lattice relaxation times and nuclear Overhauser enhancements are reported for ^{13}C nuclei in 5% *m*-cresol solutions of six polyethers containing 1,4- or 1,2-cyclohexylene rings in the chain backbone. The rings appear to move as rigid units and are opposed by low barriers to internal rotation, thus being rather more mobile than the hexose rings of polysaccharides.

Introduction

We report here some ^{13}C spin-lattice relaxation measurements on several polyethers containing cyclohexylene rings in the chain backbone[1-3] and briefly compare their revealed motional behavior to those of polymers with different backbone rings. Dynamical mechanical measurements have already been reported[4,5] for some of our polymers and are summarized in Table I. The structures of the repeat units are displayed in Figure 1.

Experimental Section

Samples of the six polyethers shown in Figure 1 were kindly supplied by Dr. J. Kops of the Instituttet for Kemiindustri. Molecular weights were estimated either from GPC or intrinsic viscosities and in all cases were sufficiently high to suppress overall tumbling (rotatory diffusion) as contributor to NMR relaxation.

Polymer solutions at about 5% (w/w) concentration were prepared in freshly distilled *m*-cresol, which, unlike other common solvents, can dissolve all six polymers at suitable temperatures. The ^{13}C NMR measurements were made at 15.04 MHz with a JEOL 60Q spectrometer. An external lock signal was employed: a small tube containing dimethyl-d_6 sulfoxide (or acetone-d_6 for polymer A) was positioned concentrically within the 5-mm tube containing the polymer solution. The method of assigning chemical shifts under these circumstances is described below. Spin-lattice relaxation times T_1 were measured with the standard technique described previously:[6] inversion-recovery with the pulse sequence $\pi - t - \pi/2 - \tau$, where the waiting time τ is chosen to be at least $5T_1$ (i.e., ca. 1 s), and with complete noise decoupling of the protons. The $\pi/2$ pulse width was 25 μs. Overhauser enhancement factors, NOE, were evaluated from ratios of the peaks

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