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THE GELATION AND CRYSTALLISATION OF AMYLOPECTIN

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ABSTRACT

A range of physical and chemical techniques, including viscometry, rheological measurements, dilatometry, turbidity measurements, X-ray diffraction, and differential scanning calorimetry, has been used to study the gelation of amylopectin. Gels form on cooling concentrated aqueous solutions to 1°. The development of gel stiffness is closely related to the association of amylopectin chains, as monitored by dilatometry and differential scanning calorimetry. X-Ray diffraction studies suggest that intermolecular association involves a crystallisation process. The association of amylopectin chains in the gel is substantial and is thermoreversible at temperatures below 100°. Heterogeneous acid hydrolysis of the gel followed by examination of the residue by gel-permeation chromatography showed that the associated regions contained branched fragments, the individual chains of which had a d.p. of 15. The combined data suggest that the amylopectin molecules associate by crystallisation of the branches with d.p. 15 to form a network.

INTRODUCTION

The retrogradation of the starch polysaccharides amylose and amylopectin from aqueous dispersions and solutions is of considerable industrial importance, particularly for the food industry where the processes can have major effects on texture and digestibility of starchy food products. Control is desirable, but at present it is largely empirical and partially effective. There is therefore a need for a greater understanding of the molecular interactions which are collectively described by the term retrogradation.

The behaviour of amylose and amylopectin might be expected to be influenced profoundly by differences in molecular structure. Whereas amylose is an essentially unbranched (1→4)-α-D-glucan, amylopectin is a highly branched macromolecule based on short (1→4)-α-D-glucan chains which are joined together through α-(1→6) branch-points. On average, there is one branch-point for every

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20–25 main-chain residues. The glucan chains are thought to have a bimodal distribution of chain length in which the most abundant species by weight have degrees of polymerisation (d.p.) of 15–20 and 50–60. The molar ratio of short to long chains varies between 3:1 and 12:1, depending on the botanical source of the starch[1]. Recently, by using improved chromatographic techniques, evidence has been obtained[2] that a polymodal distribution of chain lengths, which had only been observed for some cereal starches[3], may be more widespread than previously suspected.

The exact arrangement of chains within the amylopectin molecule is still not clear. The most widely accepted model is the "cluster model"[4–6] in which short chains, which may be multiply branched, are arranged in clusters on longer chains that are themselves linked together. A further major difference between amylose and amylopectin is molecular weight. Molecular weights of natural amyloses of up to 10^6 have been reported[5], whereas the highest estimates[7] for amylopectin are in the region of 5×10^6.

The ability of amylose to retrograde from aqueous solutions has been known for a long time[8]. The precipitate gives a weak X-ray diffraction pattern of the B-type[9]. Work on oriented fibres of amylose in the B form suggested that the X-ray intensity distribution is best explained if amylose adopts a double-helical conformation which packs antiparallel into a hexagonal array[10]. In the native state, amylopectin can also exist in crystalline form, as shown by the crystallinity of granules of waxy starches which contain solely amylopectin. Information on the parts of the amylopectin molecule involved in the crystalline regions has been obtained by heterogeneous acid hydrolysis of the granule[5]. The acid preferentially attacks the amorphous regions, leaving a more crystalline residue. Analysis of this material showed that it consisted of chains of d.p. 15, some of which were linked together; the extent of branching decreased with increasing time of hydrolysis[5]. As a result, it has been proposed[5] that the short chains of amylopectin are primarily involved in the crystalline regions of the granule.

Recent work on the retrogradation and gelation of aqueous amylose solutions[11] and starch dispersions[12] examined the time-dependence of the ordering of amylose and amylopectin molecules and its relationship to changes in the mechanical behaviour of the gel. For the gelation of amylose[11], it was proposed that gel formation arose as a result of a phase separation which produced polymer-rich and polymer-deficient regions and that, if the amylose concentration was sufficiently high, the polymer-rich regions formed an interconnected gel network. Crystallisation of the amylose, as monitored by the development of an X-ray diffraction pattern, was a secondary process which occurred in the polymer-rich regions of the gel.

Starch gels, prepared by cooling concentrated aqueous dispersions of gelatinised starch, were considered as composites in which swollen gelatinised granules, containing mainly amylopectin, were embedded in and reinforced an interpenetrating amylose gel matrix[12]. The initial development of firmness during gelation was attributed to the formation of an amylose matrix gel. The firmness of

the starch gel slowly increased with time for several days. Experimental evidence was obtained linking this change in mechanical behaviour to crystallisation of amylopectin within the gelatinised starch granule, thereby confirming earlier proposals on the possible role of amylopectin in starch retrogradation[13]. In contrast to the gelation and subsequent crystallisation of amylose, the crystallisation of amylopectin could be reversed by heating to 100°. The slow retrogradation of starch was dependent on the concentration and botanical source of the starch[14]. Of the starches examined, smooth-seeded pea starch had the greatest tendency to retrograde followed by potato, maize, and wheat starch. Experiments on the association and crystallisation of amylopectin from aqueous solutions provide data on the effect of amylopectin structure on intermolecular association. Recently, in preliminary studies[15], it has been shown that concentrated aqueous solutions gel on cooling to 1°; the gel is turbid and gives a weak X-ray diffraction pattern of the B-type crystalline modification of starch[15]. We now report a more detailed study of amylopectin gelation.

EXPERIMENTAL

Materials. — Waxy-maize starch var. Amioca was obtained from Laing National. The other starches from faba bean, normal maize, tapioca (Manihot), potato, rice, and wheat were isolated by an aqueous extraction procedure[16]. Waxy-maize amylopectin was obtained by dissolving the starch granules in aqueous 90% Me_2SO followed by precipitation of the amylopectin with ethanol. The other amylopectins were obtained after precipitation of amylose and intermediate material with thymol[17].

Pullulanase (EC 3.2.1.4.1) from *Enterobacter aerogenes* was obtained from Hayashibara, Okayama, Japan, and sweet-potato beta-amylase (EC 3.2.1.2) from Koch–Light Laboratories. Maize beta-limit dextrin was prepared by treatment[18] of maize-starch amylopectin with beta-amylase. Debranching of maize amylopectin and amylodextrins was performed by using pullulanase in aqueous 20% Me_2SO as described by Mercier and Kainuma[19], and the product was diluted ten-fold with water and then freeze-dried.

Preparation of gels. — Concentrated amylopectin solutions were prepared by dissolving the amylopectin with gentle agitation in boiling, oxygen-free water containing 0.02% of sodium azide as a preservative. Hot solutions were rapidly quenched to 1° to initiate gelation.

Shear modulus measurements. — Network formation was monitored by following the development of the shear modulus G', using a Rank Brothers' Pulse Shearometer as described by Ring and Stainsby[20]. The stress–strain behaviour of the amylopectin gels, at strains <0.1, was investigated by using a modified Saunders and Ward tube as described elsewhere[21].

Viscometry. — The dependence of specific viscosity on concentration, over a wide range of concentrations, was measured by using Ubbelohde suspended-level viscometers at 25°.

Turbidity measurements. — The development of turbidity with time during the gelation of amylopectin was monitored by using a Pye Unicam SP800 spectrometer (640 nm, 0.1-cm pathlength). The turbidity of the solutions and gels was measured relative to that of water.

Dilatometry. — The isothermal volume change at 1° which occurred during the gelation of amylopectin was determined as described elsewhere[11].

Differential scanning calorimetry. — D.s.c. studies were performed as described elsewhere[12], using a Perkin–Elmer DSC-2B instrument. High temperature scans (20–190°) were carried out at a scanning rate of 3°/min, using a SETARAM DSC-111 with water as the reference material. The gel containing 12–15 mg of polysaccharide was hermetically sealed in a steel pan and allowed to equilibrate for 24 h at 20° prior to analysis.

Lintnerisation. — The heterogeneous acid hydrolysis of the starches and the amylose and amylopectin gels in 2.2M HCl at 35° was performed as described elsewhere[22]. The insoluble residue after prolonged hydrolysis was isolated by centrifugation and washed free of acid and fragments of low molecular weight. Samples (~20 mg) of the residue were then dissolved in 1 mL of M KOH at 4°. The soluble amylodextrins were fractionated on a column (1000 × 1.6 cm) of HW-50S (Merck), by upward elution with 0.1M KOH at a flow rate of 7.8 $mL.h^{-1}.cm^{-3}$. The column (V_0 55.6 mL; V_t 113.4 mL) was calibrated by using malto-oligosaccharides of known d.p. obtained commercially or prepared by debranching of native waxy-maize amylopectin[5].

The elution of carbohydrate was monitored by differential refractometry, using a Waters R-401 refractive index detector which had been calibrated with glucose solutions. All other determinations were performed as described previously[23].

X-Ray diffraction. — Wide-angle X-ray patterns were recorded photographically using a flat plate camera. Time-dependent changes in crystallinity were monitored by following the development of the (100) reflection (*d*-spacing 1.6 nm), characteristic of the B-type crystalline modifications of starch, using a Kratky small-angle X-ray scattering camera. The diffracted intensity was recorded at selected angles by scanning the appropriate angular range. Scans were repeated at appropriate time intervals. The crystallinity was assessed by measuring the integrated intensity of the diffraction peak above the background intensity of the non-crystalline polymer and solvent. The X-ray wavelength was the Cu–K_α line at 0.154 nm.

RESULTS AND DISCUSSION

Physical studies. — Waxy-maize amylopectin was used for most of the experiments. The iodine binding capacity (at an extrapolated zero concentration of free iodine) of the preparation was <0.2 mg of iodine bound per 100 mg of polysaccharide. The preparation was therefore considered to be >98% amylopectin. The intrinsic viscosity of the amylopectin in aqueous solution at 20° was 80 $mL.g^{-1}$, which

is comparable to data on other amylopectins[7], and indicates that the preparation was of high molecular weight.

Preliminary experiments on the storage of concentrated aqueous solutions of waxy-maize amylopectin at 1–20° showed that, with decreasing temperature, polymer aggregation, as indicated by an increase in turbidity, was more evident. At temperatures below 5°, solutions of concentration >10% formed gels. Gelation was more rapid at the lower temperatures and, for this reason, experiments were conducted at 1°. Even at this temperature, gelation was slow, the modulus of a 10%

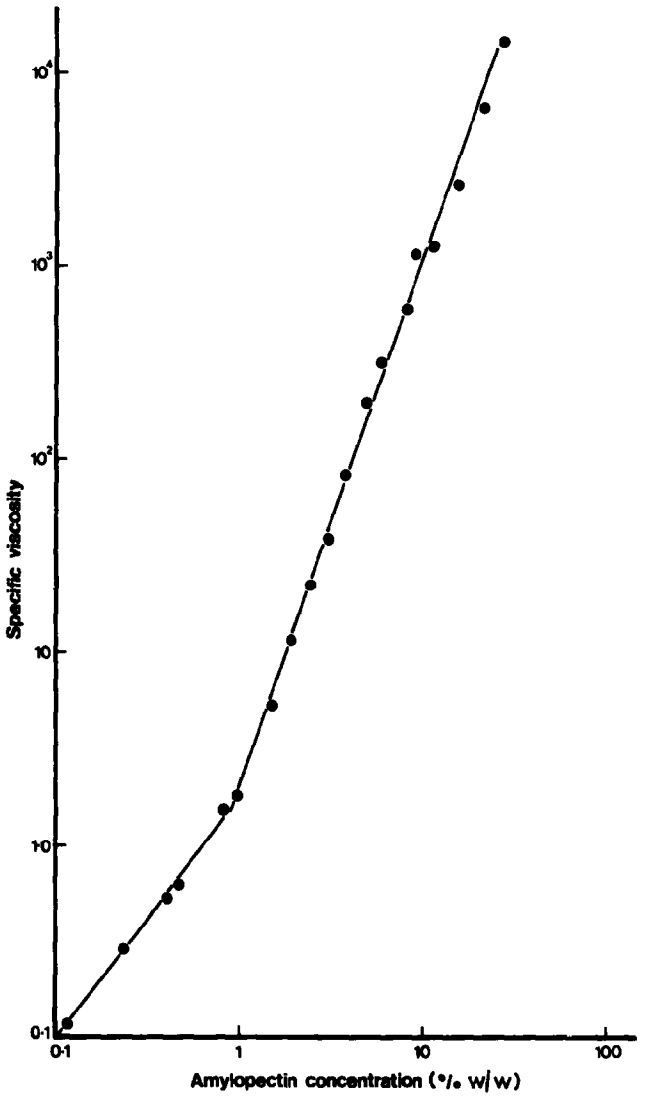

Fig. 1. Double logarithmic plot of specific viscosity *versus* amylopectin concentration in the range 0–25% at 60° in aqueous solution.

gel taking several weeks to approach a limiting value. With increasing concentration, gelation was more rapid.

It is of interest to characterise the concentration regime in which gelation occurs in order to assess whether the polymer chains are in the dilute or semi-dilute region. An experimentally convenient method is to examine the dependence of specific viscosity on concentration. For amylose[11] solutions, there is a linear relationship between specific viscosity and concentration below a threshold concentration C^*. Above this concentration, as chain entanglements make a contribution to the observed viscosity, the viscosity becomes dependent on the square of the polymer concentration. Gelation only occurs above C^*, which for amylose of molecular weight 5×10^5 was ~1.5%. The branching of amylopectin might be predicted to have important concentration-dependent effects on viscosity. At a fixed molecular weight, the branching of a polymer chain reduces the hydrodynamic volume with the result that C^* is shifted towards higher values than for a linear chain. Also, above C^*, the presence of chain branches strongly hinders the reptation of the polymer molecule, such that the viscosity of the solution is enhanced and the relaxation time of the entangled polymer solution becomes longer. Fig. 1 shows a double logarithmic plot of specific viscosity *versus* concentration for waxy-maize amylopectin in aqueous solution at 20°. At concentrations below 0.9%, there was a linear relationship between specific viscosity and concentration (C). In the concentration range 0.9 to 25%, the dependence changed to $C^{2.7}$. Thus, the overlap concentration C^* occurs in the region of 0.9%. At 1°, gelation starts to occur at concentrations of >10% where the amylopectin chains are heavily entangled. In this respect, the gelation behaviour of amylopectin is quite different to that of amylose.

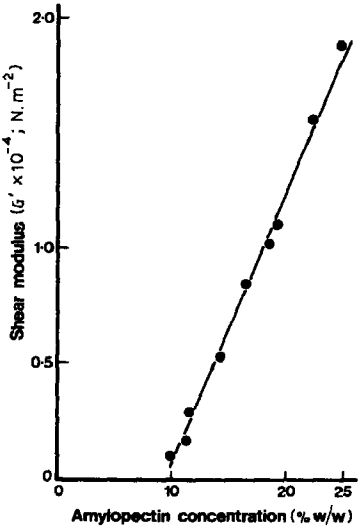

Fig. 2. Graph of shear modulus, G', *versus* concentration for waxy-maize amylopectin gels.

Concentrated amylopectin solutions were viscoelastic. The amylopectin gels behaved as Hookean solids at strains <0.1. Small applied static deformations quantitatively and quickly recovered upon removal of the applied stress.

Fig. 2 shows a plot of shear modulus *versus* concentration for waxy-maize amylopectin gels in the concentration range 10–25% after storage for 6 weeks at 1°. In this range, there was a linear relationship between modulus and concentration. This is in marked contrast to the observed 7th power dependence of modulus on concentration for amylose gels[24].

During gelation, the amylopectin solution changed from a viscoelastic material, which flowed under a constant small applied stress, to a gel. Fig. 3 shows the changes in G' with time during the gelation of a 20% amylopectin solution at 1°. The curve has a sigmoidal shape, with the modulus approaching a limiting value (of G'_∞) of 1.15×10^4 N.m^{-2} after 30–40 days. The value of G'_∞ obtained at 200 Hz was in good agreement with the modulus obtained under static loading conditions.

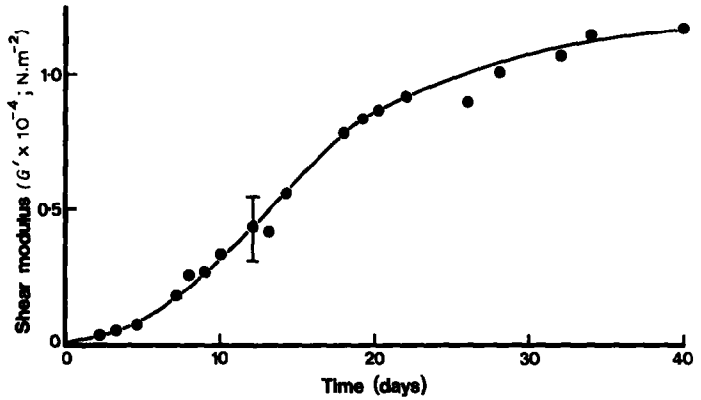

Fig. 3. Graph of shear modulus, G', as a function of time during the gelation of a 20% amylopectin solution at 1°.

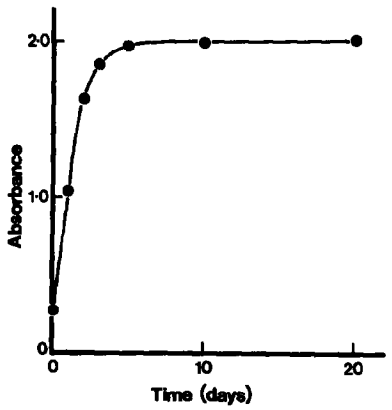

Fig. 4. Graph of turbidity as a function of time during the gelation of a 20% amylopectin solution at 1°.

Several techniques, including turbidimetry, dilatometry, and differential scanning calorimetry (d.s.c.), were used to monitor the association of amylopectin chains during gelation. Amylopectin gels became turbid and finally opaque during gelation, the increase in turbidity indicating that changes in refractive index distribution and hence of density distribution occur within the sample. Fig. 4 shows the increase in turbidity with time during the gelation of a 20% amylopectin solution. The turbidity approached a limiting value after 4–5 days; during this time, the modulus, G', had shown only a small increase (Fig. 3). The opacity indicates that the precursors of gelation are aggregates of amylopectin molecules. Quantitative analysis of the data is difficult because such experimental problems as multiple scattering are likely to affect the observed turbidity at quite early stages of gelation.

The other techniques give more information on the extent of association between polymer chains. Dilatometry has been used to study phase transitions in polymer diluent systems[25] and in the study of the gelation of amylose[11] and gelatin[26]. The volume changes observed are indicative of changes in strength, number, and symmetry of interactions of the molecules. Fig. 5 shows the isothermal volume change as a function of time during the gelation of a 20% amylopectin solution at 1°. The observed volume changes were positive and approached a limiting value, ΔV_∞, after 30–40 days. The magnitude of the change (1.02×10^{-6} $m^3.mol^{-1}$ anhydro hexose) was much larger than that observed for amylose (0.4×10^{-7} $m^3.mol^{-1}$ anhydro hexose).

Amylopectin gels are thermo-reversible, and gel melting, as indicated by a decrease in modulus, occurred in the range 40–60°. It was possible to follow gel melting by d.s.c. Melting of the gel is accompanied by a loss of turbidity.

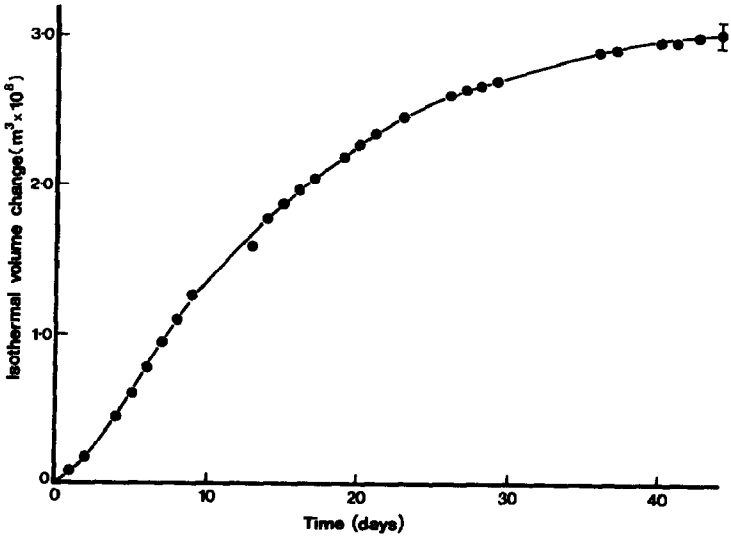

Fig. 5. Graph of isothermal volume change as a function of time during the gelation of a 20% amylopectin solution at 1°.

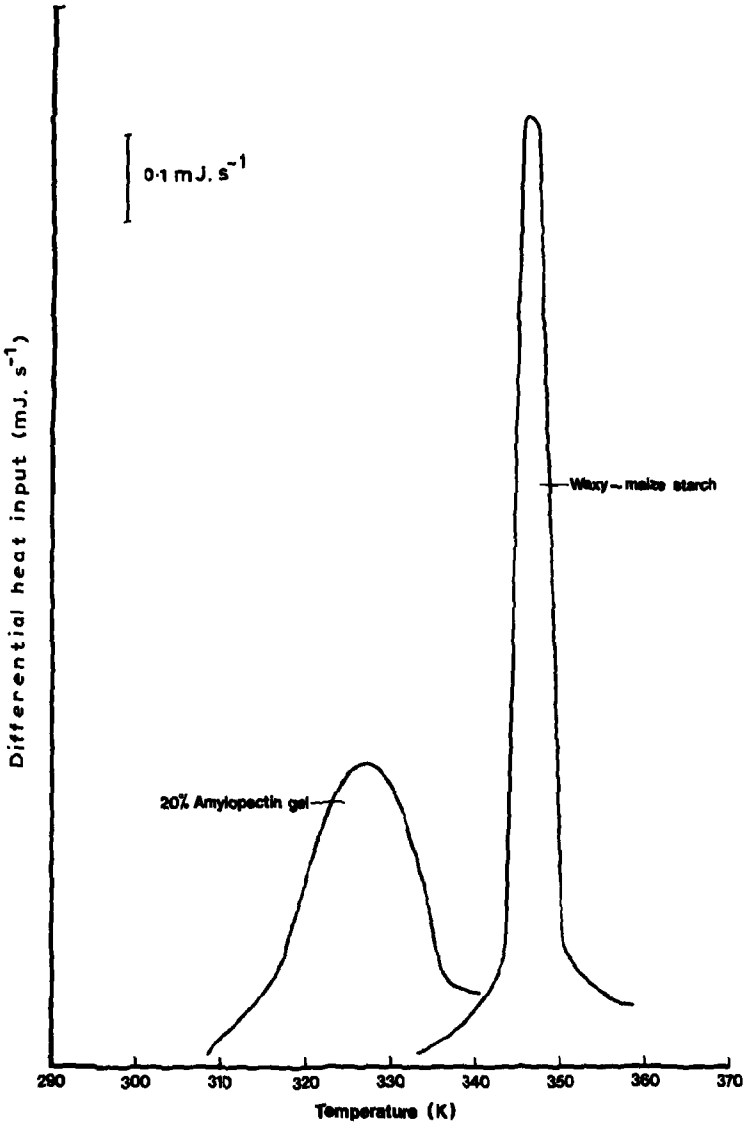

Fig. 6. Graph of differential heat flux as a function of temperature for the gelatinisation of an aqueous 20% waxy-maize starch dispersion and a 20% waxy-maize amylopectin gel.

Fig. 6 shows typical thermograms for the dissolution of a 20% waxy-maize amylopectin gel stored for 4 weeks at 1° and also the gelatinisation transition for a 20% dispersion of waxy-maize starch granules. Endothermic peaks are obtained in each thermogram. For the gel, the mid-point of the transition occurred at 54° and spanned 23°. The gelatinisation endotherm was sharper, occurred at 75°, and spanned 6°. The measured enthalpies of the transitions were similar at 15 mJ/mg of

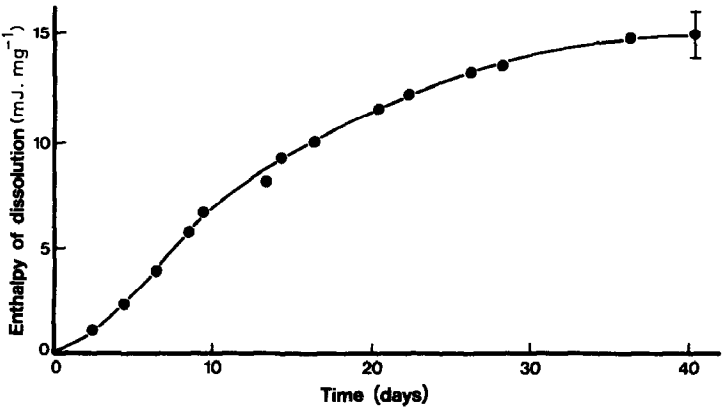

Fig. 7. Graph of enthalpy of gel dissolution, H, as a function of time during the gelation of a 20% amylopectin solution at 1°.

polysaccharide for the gel and 15.4 mJ/mg for the starch. Fig. 7 shows the changes in enthalpy of gel dissolution (ΔH) as a function of time during the gelation of a 20% amylopectin solution at 1°; ΔH slowly increased with time, approaching a limiting value after 30–40 days. The width of the transition and the temperature of the mid-point did not significantly change during this time. The quantities G', ΔH, and ΔV all develop over a similar time-scale. The curves of $\Delta V - t$ and $\Delta H - t$ can, with appropriate scaling, be superimposed. The curve of $G' - t$ can only be superimposed during the later stages of gelation. During the early stages (<10 days), the development of the modulus lags behind the development of the intermolecular association.

X-Ray diffraction data can be used to assess the role of crystallisation on intermolecular association and gelation. The formed gels showed diffraction patterns characteristic of the B-type crystalline modification of starch. As reported elsewhere[15], the crystallinity monitored by X-ray diffraction can be melted out on heating the gels. Fig. 8a shows the increase in crystallinity I, monitored by X-ray diffraction, during gelation of a 20% amylopectin solution at 1°. Measurements carried out at different concentrations of amylopectin showed that the level of crystallinity increased linearly with increasing concentration of polymer. By appropriate choice of the constants (k_I and k_H), the curves $k_I I(t)$ and $k_H \Delta H(t)$ can be superimposed, within the limits of experimental accuracy, at each polymer concentration. These results suggest that the d.s.c. studies monitor a thermoreversible crystallisation which occurs from intermolecular association of amylopectin. At short storage times, the development of the network structure, as monitored by measuring the shear modulus, lags behind development of the crystallisation. X-Ray diffraction will monitor amylopectin aggregate precursors below the critical size for gelation.

The d.s.c. data contain a large contribution due to the melting of amylopectin crystallites. At 20°, the glassy and crystalline forms of D-glucose have heats of

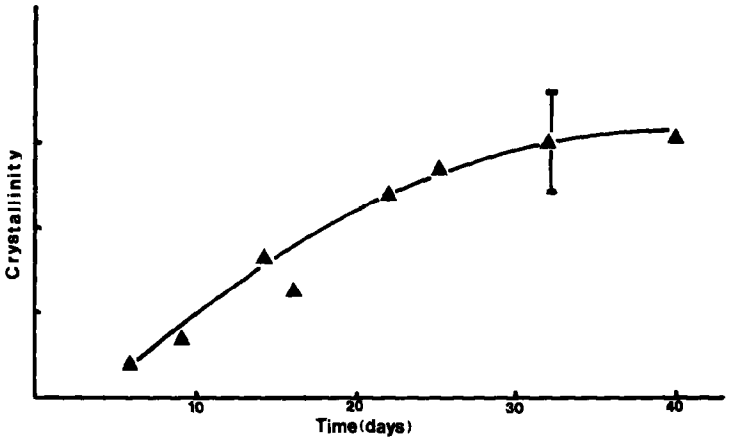

Fig. 8. Graph of crystallinity, monitored by X-ray diffraction (arbitrary units).

solution which are[27] negative and positive, respectively. Short-chain amylose glasses dissolve in water with the evolution of heat[28]. A 20% dispersion of B-type crystalline spherulites prepared from short (d.p. 20) amylose chains[29] shows an enthalpy of melting of 42.1 mJ/mg of polysaccharide with a mid-point transition of 74°. The dissolution endotherm for the amylopectin gel is approximately one third of this in size. If the spherulites are assumed to be perfectly crystalline, then this comparison would suggest that the level of crystallinity within the gel is at least 30%. However, the endotherms for the gel are much broader than those obtained for the spherulites, indicating that, although the level of crystallinity is high, the crystals show a wide range of size and/or crystal perfection. Many synthetic polymers that are capable of existing in crystalline form show similar behaviour, in that they form non-equilibrium gels as a result of the association of polymer chains in a poorly crystalline phase. Similar observations have been made for the gelation of malto-oligosaccharides[30].

The gelation of amylopectins from other botanical sources was also examined. With sufficiently concentrated solutions, all amylopectins produced elastic gels on storage at 1°. Potato and tapioca amylopectin gels were translucent, whereas cereal and faba-bean amylopectins formed turbid gels. After storage for 1 month at 1°, the shear modulus of 25% gels ranged from 8.4×10^2 $N.m^{-2}$ for tapioca amylopectin to 2.17×10^4 $N.m^{-2}$ for a wheat amylopectin (Table I). The tuber (tapioca and potato) amylopectins produced the softest gels. In all cases, the gels were thermo-reversible at temperatures below 100°. On d.s.c. analysis, each gel gave a relatively broad endothermic peak with the mid-point of the transition ranging from 54 to 60° (Table I). The enthalpies of transition ranged from 3.1 to 7.2 mJ/mg of polysaccharide. There appears to be no simple relationship between the observed enthalpy of transition and the shear modulus of the different gels at a fixed concentration.

TABLE I

CHARACTERISTICS OF GELS MADE WITH POTATO AMYLOSE AND AMYLOPECTINS OF VARIOUS ORIGINS

Polysaccharide		*Concentration (% w/w)*	*Shear modulus, G' (N.m.$^{-2}$)*	*Temperatures[a] (°) of melting peak*			*Enthalpy change (mJ/mg of polysaccharide)*
				T_1	T_2	T_3	
Potato amylose		12	6711.4×10^2	143.2	153.1	164.2	9.4
Amylopectin							
Faba bean		25	149.9×10^2	40.1	59.5	71.1	4.8
Maize	native	25	129.9×10^2	45.3	58.7	64.7	6.8
	P[b]	25	345.3×10^2	47.7	60.2	67.5	6.4
	β[c]	25	Not detected	Not detected	Not detected	Not detected	Not detected
Potato		25	13.9×10^2	42.3	59.3	73.2	4.9
Rice		25	73.2×10^2	40.7	56.7	68.7	6.0
Tapioca		25	8.4×10^2	41.7	57.5	65.8	3.1
Wheat		25	217.8×10^2	44.2	54.2	64.0	7.2

[a] T_1, Beginning temperature; T_2, temperature with maximum enthalpic change; T_3, ending temperature. [b] P, Debranched amylopectin. [c] β, Beta-limit dextrin of native amylopectin.

Structural studies. — Further work was carried out in order to establish which regions of the amylopectin molecule were involved in interchain association and crystallisation. Treatment of waxy-maize amylopectin with beta-amylase, to produce a beta-limit dextrin, destroyed its ability to gel; 25% solutions remained clear on storage at 1° for 1 month and the endothermic transition was absent (Table I). Linear dextrins (beta-amylolysis limit, 100%) prepared by debranching maize amylopectin with pullulanase did form gels at high concentration. The shear modulus of a 25% linear-dextrin gel stored for 1 month at 1° was 3.45×10^4 N.m^{-2} and an endotherm of enthalpy 6.4 mJ/mg of polysaccharide at a mid-point of 60° was observed by d.s.c. Amylose gels do not melt on heating to 100°. However, using a Setaram DSC-111, it was possible to demonstrate the presence of an endothermic peak at 153° (enthalpy, 9.4 mJ/mg of polysaccharide) for a 12% potato amylose gel (Table I). Amylopectin gels showed no such transition at these higher temperatures.

The inability of the beta-limit dextrin to gel favours the view that the exterior amylopectin chains crystallise during gelation. To obtain information on the lengths of polymer chains involved in these crystallites, the gel was subjected to heterogeneous acid hydrolysis, which has been used[5,31] to erode preferentially the amorphous

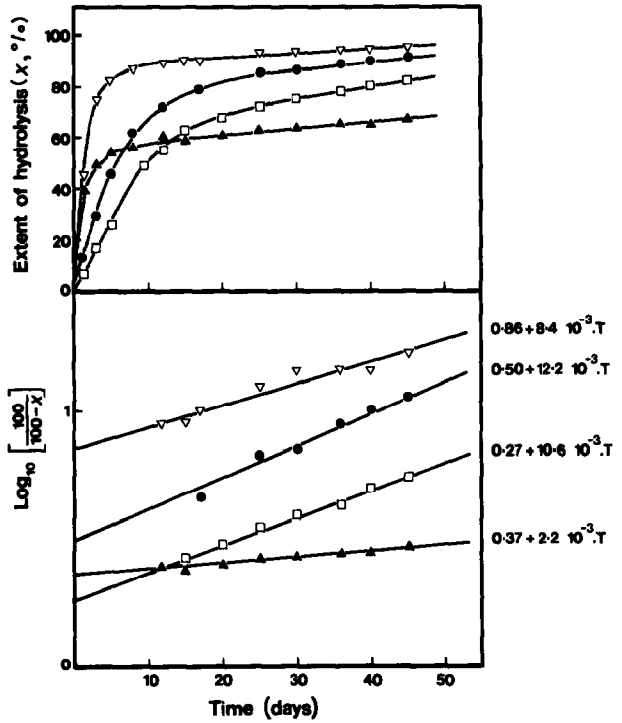

Fig. 9. Graph of hydrolysis as a function of time for amylose (▲) and amylopectin (▽) gels, and for potato (□) and waxy-maize (●) starches. Expressed as (*a*) extent of hydrolysis (x) *versus* time and (*b*) as $\log_{10}[100/(100 - x)]$ *versus* time. Degradation rates are shown by the side.

regions of semi-crystalline starch solids. In this way, it has been possible to determine the structure of the polymer segments which form the crystalline regions in starch granules[5], amylose-V complexes[32], and retrograded amylose[32]. Fig. 9 shows a plot of the extent of hydrolysis (x) as a function of time (t) for the hydrolysis of potato and waxy-maize starch granules, a 25% maize amylopectin gel, and a 12% potato amylose gel. The curves show an initial high rate of hydrolysis, lasting ~3 days for the gels and ~10 days for the granular starches, followed by a much lower rate. During the initial phase, at least 50% of the material is hydrolysed. An estimate of the more easily degraded fraction as a percentage of the total polysaccharide can be obtained by extrapolating plots of $\log_{10}[100/(100 - x)]$ *versus* time to zero time (Fig. 9); the plots are linear. The proportion of the easily degradable material is higher for the amylopectin gel (86%) than for waxy-maize starch (68%), potato starch (46%), and the amylose gel (57%).

The rate of degradation of the less easily degraded material, obtained from the slope of $\log_{10}[100/(100 - x)]$ *versus* time plots was broadly similar for the amylopectin gel (8.4×10^{-3} day^{-1}), potato starch (10.6×10^{-3} day^{-1}), and waxy-maize starch (12.2×10^{-3} day^{-1}), but much lower for the amylose gel (2.2×10^{-3} day^{-1}). Acid-resistant residues representing 6% of the polysaccharide of the amylopectin gel, 23% of the amylose gel, 9% of the waxy-maize starch, and 18% of the potato starch were isolated by centrifugation after washing to remove soluble carbohydrate and acid. The residues were dissolved in M KOH and subjected to gel-permeation chromatography. Fig. 10 shows the elution profiles which were obtained as weight distributions. The potato starch amylodextrin (λ_{max} for I_2 complex, 535 nm) gave a major peak at d.p. 14 (V_e 90 mL) with a shoulder at higher molecular weight. After pullulanase treatment, the shoulder disappeared but the position of the maximum did not change. The waxy-maize starch amylodextrin (λ_{max} for I_2 complex, 547 nm) gave a bimodal elution profile with modal values of d.p. 23 (V_e 83 mL) and d.p. 14 (V_e 91 mL). On debranching with pullulanase, a major peak of d.p. 15 was obtained. In both cases, the pullulanase-debranched material was linear as judged by its quantitative hydrolysis by the endo-acting enzyme beta-amylase. These results indicate that chain association in starch granules extends over ~15 residues and involves the branched polysaccharide amylopectin. These conclusions are in agreement with previously published work[5,33,34]. The residue after hydrolysis of the amylose gel (λ_{max} for I_2 complex, 576 nm) gave a single, rather broad peak of d.p. 53 (V_e 67 mL), starting at d.p. 90 (V_e 55 mL) and ending at d.p. 10 (V_e 95 mL). Treatment of the material with pullulanase did not significantly affect the elution profile or the beta-amylolysis limit of 98%. In a similar study of potato amylose that was allowed to retrograde from a more dilute solution (0.3%), Jane and Robyt[32] found that the acid-resistant amylose fragment had $d.p._n$ 32. In the present study, the calculated $d.p._n$ of the amylose residue was 30, which shows that, on precipitation from dilute or concentrated aqueous solutions, the amylose chains associate over similar lengths.

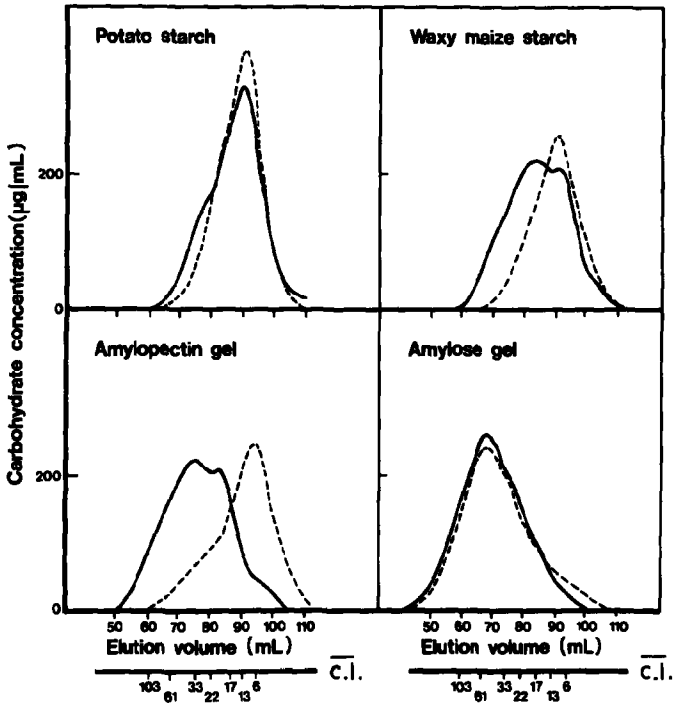

Fig. 10 Elution profiles on MW-50S of amylodextrins (——) and debranched amylodextrins (-----) from amylopectin gel, waxy-maize starch, potato starch, and amylose gel.

The acid-resistant residue from the amylopectin gel (λ_{max} for I_2 complex, 549 nm) gave a bimodal elution profile with modal values of d.p. 45 (V_e 71 mL) and 24 (V_e 78 mL), and a small shoulder present at d.p. 15 (V_e 90 mL). After debranching with pullulanase, a single major peak of d.p. 14 (V_e 91 mL) was obtained with a shoulder of d.p. 30–40 (V_e 30–80 mL). The beta-amylolysis limit of pullulanase-debranched material was 100%. The amylodextrin prepared from the amylopectin gel therefore consists of single or multiply branched molecules, the chains of which have $d.p._w$ 15. Amylodextrins with similar structures were obtained after heterogeneous acid hydrolysis of gelatinised maize and waxy-maize starches which had been subjected to freeze–thaw cycles[35]. These studies show that, in the starch granule and in the amylopectin gel, the length of chain over which association and crystallisation occurs is relatively short.

For current models of amylopectin fine-structure, these branched fragments could either derive from a cluster with a significant number of branches (d.p. 15) near the potential reducing-group of the fragment or from two clusters linked on the same chain of d.p. 45. The observed λ_{max} of the iodine–amylodextrin complex of 549 nm is at a longer wavelength than expected for chains of d.p. ~15, suggesting the presence of longer unsubstituted chains, and the second structure is therefore more likely. The presence of less acid-susceptible α-(1→6) linkages could be

explained by their involvement, near crystalline areas, with conformational restrictions preventing the mobility of the glycosidic bond. A consequence of the proposed antiparallel stacking of the (1→4)-α-D-glucan double-helices in the B-type crystalline form is that different clusters would need to interdigitate before crystallisation could occur, by inter- or intra-molecular mechanisms.

For polymers of low molecular weight or polymer fragments, the temperature of dissolution of crystallites in a solvent is profoundly influenced by chain length. For the amylopectin, the evidence obtained has shown that interchain association involves chain segments of $d.p._w \sim 15$, whereas the amylose association is more extensive and involves chain segments of $d.p._w \sim 50$. The temperature of dissolution of amylose therefore should be much higher. This is in agreement with the observed temperatures of dissolution of the amylose and amylopectin gels of 153° and ~59°, respectively.

This study confirms that the gelation from aqueous solution is a general property of amylopectins.

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