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Microstructure of amylose gels

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The aim of this investigation was to study the structural characteristics of amylose gels (2–8% w/v) at different scales of organization. Native gels have been studied by electron microscopy, mild acid hydrolysis, differential scanning calorimetry and size-exclusion chromatography. A model for organization of the polysaccharide within the gel structure is proposed.

Amylose gels exhibited a macroporous structure (mesh size 100–1000 nm) containing filaments 20 ± 10 nm wide. The filaments resulted from the association of segments of amylose chains ($26 < DP_n < 31$; $56 < DP_w < 73$) which were orientated obliquely to the filament axis. These amylose fragments were partially organized in a B-type crystalline array.

Amylose gels also contained an amorphous fraction consisting of 18–33% of the polysaccharide. This fraction was easily degraded by acid hydrolysis. The amorphous fraction was described as dangling chains ($6 < DP < 30$) located in the macroporous structure. It was mainly responsible for the hydrodynamic behaviour and the porosity of native amylose gels.

The limit between the associated and amorphous regions of the gel was not perfectly defined and an intermediate zone which may recrystallize upon acid hydrolysis was identified.

INTRODUCTION

Knowledge of the network organization and related properties of biopolymer gels is of interest in biology and for industrial applications of these materials. Starch occurs as a mixture of two polymers of D-glucose, amylose and amylopectin. Starch has long been used to texturize and control mechanical properties of many products, particularly in the food industry through its gelling behaviour (Zobel, 1984; Swinkels, 1985; Morris, 1990). Recently the need for three-dimensional matrices with controlled release properties for agrochemical and pharmaceutical uses (Wing *et al.*, 1987; Trimnell & Shasha, 1988; Wing *et al.*, 1988; Reed *et al.*, 1989; Heller *et al.*, 1990; Levy & Andry, 1990) has increased interest in understanding starch gelation and gel microstructure. As starch is also a nutrient, control of its digestibility may be of interest in the treatment of diabetes and obesity in man (Björck *et al.*, 1986, 1987). Starch gels are partially resistant to amylolytic hydrolysis, although the reasons for this are not fully understood (Bornet *et al.*, 1989; Colonna *et al.*, 1990). In particular, the structural features of the gel which restrict the action of the starch degrading enzymes, have not yet been sufficiently characterized.

The molecular mechanism of gel formation of starch (Miles *et al.*, 1984; Ring, 1985; Ring & Stainsby, 1985; Mestres *et al.*, 1988*a*), amylose (Miles *et al.*, 1984, 1985*a*) and amylopectin (Ring *et al.*, 1987; Kalichevsky *et al.*, 1990) has been studied. Opaque gels are obtained upon cooling of concentrated starch aqueous dispersions as a result of phase separation occurring in the water–amylose–amylopectin system, which is followed by a nucleation leading to the development of a 'thin' three-dimensional amylose network (Kitamura *et al.*, 1984; Doublier & Chopin, 1989; I'Anson *et al.*, 1988; Gidley &

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Bulpin, 1989). At this stage, elementary junction zones are locally established between the macromolecules. These should adopt locally a left-handed, parallel-stranded double helical conformation (Imberty & Perez, 1988). On ageing, starch gels develop a B-type crystallinity (Katz, 1930; Miles *et al.*, 1984) from an aggregation process resulting in parallel arrays of the elementary junction zones (Imberty & Perez, 1988). Although the mechanism of starch gelation has been studied, relatively little has been published on the microstructure of starch networks. The composite structure of starch gels resulting from amylose–amylopectin incompatibility (Kalichevsky & Ring, 1987; Orford *et al.*, 1987; Mestres *et al.*, 1988*b*; Leloup *et al.*, 1991) may be studied by examining the microstructure of mixed amylose and amylopectin gels as a function of composition and at a length scale of several micrometers using light microscopy.

Amylose gels are obtained by cooling semi-dilute solutions of amylose ($\geqslant$ 15 mg/ml) (Ellis & Ring, 1985; Miles *et al.*, 1985*a*), whereas aggregates are formed in the dilute regime (<15 mg/ml) (Jane & Robyt, 1984; Clark *et al.*, 1989). The mechanical and rheological properties of amylose gels have been studied (Ring, 1985; Clark *et al.*, 1989). In addition, aspects of microstructure such as porosity (Leloup *et al.*, 1990) and crystallinity (Gidley, 1989; Cairns *et al.*, 1990) have been probed. It is now well established that amylose gels are poorly crystalline structures composed largely of an amorphous fraction. So far, these studies have not described the overall organization of the macromolecules into amorphous and crystalline regions and their interconnection. In a study on retrograded amylose obtained on preparation from solution of low concentration (3·5 mg/ml), Jane and Robyt (1984) proposed a molecular model for the amylose aggregates of crystalline double helical regions that are ~10 nm long, interspersed with amorphous regions.

The purpose of this study was to investigate, using electron microscopy and acid hydrolysis methods, the microstructural organization of amylose gels, at a length scale of tens of nanometres. A molecular model for the gel structure is proposed.

MATERIALS AND METHODS

Materials

Pea starch was prepared from smooth-seeded peas (*Pisum sativum*, cultivar Filby) by aqueous extraction (Adkins & Greenwood, 1966). Amylose was then extracted from pea starch by leaching at 70°C and purified by *n*-butanol precipitation. The average degree of polymerization of pea starch amylose ranged from 2000 to 3000 (Ring *et al.*, 1985). Amylose solutions of concentration ranging from 2 to 8% (w/w) were obtained by heating the aqueous *n*-butanol complex and evaporating the *n*-butanol in a heated nitrogen stream (Miles *et al.*, 1984). Amylose gels were prepared by rapid quenching to room temperature and were kept 4 days at 1°C in order to reach a steady-state structure. Amylose gel concentration was determined from the dry weight (2 h, 120°C).

Short linear α-D-(1,4)-glucan chains (degree of polymerization $DP_n \sim 15$) were prepared by mild acid hydrolysis at 35°C of potato starch as previously described (Robin *et al.*, 1975). Glucose was purchased from Sigma Chemicals Co. (St Louis, USA).

Electron microscopy study

Amylose gels (4·7 and 7·8%) were cast in small syringes ($\phi \sim 4$ mm). Thin slices (0·1 mm) of gel were freshly cut and mounted on the specimen support of a MedVac Cryopress (St Louis, USA). The sample was dropped under gravity on to a pure copper block cooled with liquid helium to produce a thin (~10 μm) layer of very rapidly quenched, vitreous ice (Heuser, 1981). Frozen samples are stored in liquid nitrogen until transfer to a Balzers freeze-fracture unit (BAF400D). Here the frozen surface was scraped clean at −115°C and the exposed surface etched by raising the temperature to −100°C for about 15 min. The etched surface is replicated by rotary shadowing with platinum at an angle of 20° followed by carbon at an angle of 50° (McCann *et al.*, 1990). The replica was cleaned in 70% sulphuric acid, washed and supported on a plastic filmed copper grid, before being examined in a JEOL1200EX electron microscope. Micrographs were printed onto reverse-contrast paper (Kodak Graphic Arts).

Mild acid hydrolysis

Amylose gel particles were prepared by passing gel fragments through a 250 μm mesh sieve. A suspension containing 100 mg (d.b.) of an amylose gel in 20 ml of hydrochloric acid (2·2N) was kept for 35 days at 35°C. Aliquots (0·25 ml) were withdrawn daily, neutralized with potassium hydroxide (2·2N) and centrifuged. The amount of soluble α-glucans was quantified by the sulphuric orcinol method (Tollier & Robin, 1979). The extent of hydrolysis was expressed as soluble polysaccharide formed as a percentage of the initial amount of α-D-glucans present in the gel. The residues recovered after 35 days hydrolysis were thoroughly washed with distilled water and quickly frozen in liquid nitrogen and lyophilized.

Chromatographic analysis

Residue (5 mg/ml) was dissolved in 0·1N KOH at room temperature. A sample of this solution (50 μl) was chromatographed on a Pharmacia Superose 12 TM column, 30 cm × 1 cm (Uppsala, Sweden) eluted with 0·1N KOH at a flow rate of 20 ml/h at 20°C. Fractions

(*i*) were collected every minute and analyzed for carbohydrate (C_i) by the orcinol sulphuric method. The column parameters, V_0 (void volume) and V_t (total volume) were determined with amylose and glucose, respectively. Each fraction was characterized by its K_{av}:

$$K_{av} = (V_e - V_0)/(V_t - V_0) \quad (1)$$

Linear chains of α-D-glucans of known *DP* prepared by acid hydrolysis of potato starch were also used to calibrate the column. α-Glucan concentation in the eluant was determined by the sulphuric orcinol procedure (Tollier & Robin, 1979), and the reducing sugar value of the eluant was determined (Hizukuri *et al.*, 1981). The degree of polymerization (*DP*) of each fraction was then calculated. A calibration curve of the *DP* as a function of K_{av} was obtained:

$$\log (DP) = 3{\cdot}60 - 2{\cdot}91\, K_{av} \; (R = 0{\cdot}99) \quad (2)$$

which enabled calculation of average degrees of polymerization, DP_n and DP_w, and polydispersity, p, of the hydrolysis residues as follows:

$$DP_n = \Sigma C_i/\Sigma (C_i/DP_i) \quad (3)$$

$$DP_w = \Sigma (C_i\, DP_i)/\Sigma C_i \quad (4)$$

$$p = DP_w/DP_n \quad (5)$$

where C_i and DP_i are the α-glucan concentration and the degree of polymerization, respectively.

Thermal analysis

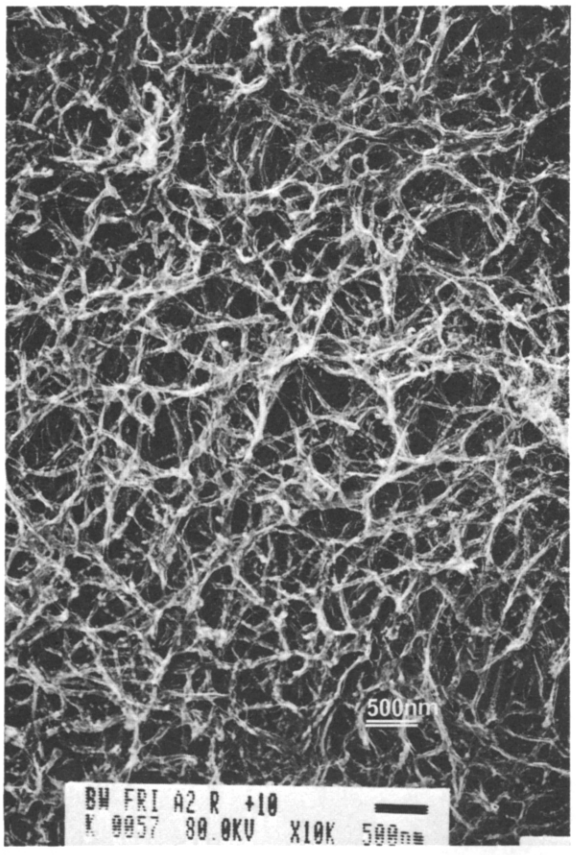
Thermal analysis was performed on native, and hydrolyzed, lyophilized gels using a Setaram 111 differential scanning calorimeter. Samples of ~10 mg were accurately weighed and added to 110 mg of water. Samples were heated from 30 to 160 °C at a constant rate of 3 °C/min, comparing them to a reference containing 120 mg of water. The enthalpy change for a melting transition was calculated by integrating the endothermic peak between the initial and final temperatures of melting (Mestres *et al.*, 1988*a*).

RESULTS

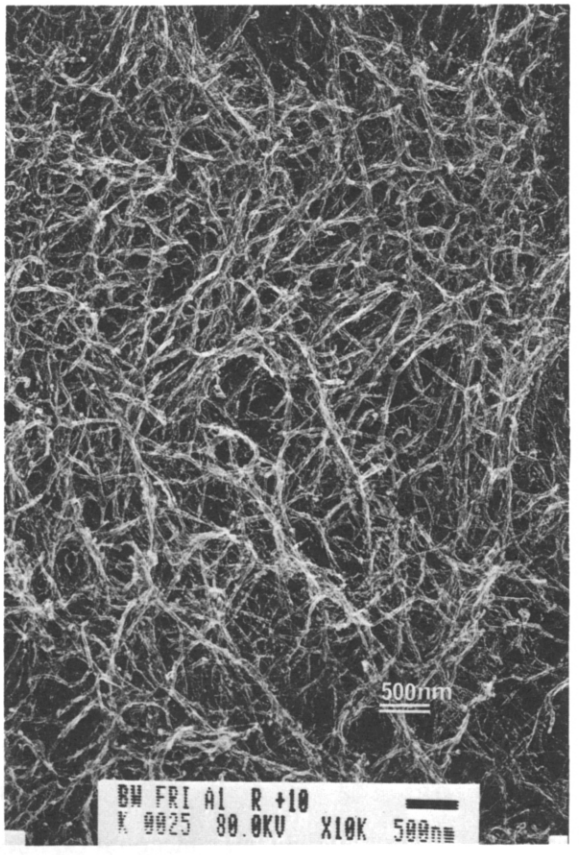
The quick-freeze technique is a well adapted method for preserving the microstructure of highly hydrated physical gels involving low energy bonds (Favard *et al.*, 1989). However, the depth which ultrarapid freezing may reach is limited by the growth of ice crystals as the distance from the surface of the specimen increases. Intact preservation of stuctures thus occurs only in superficial zones of the specimen. Favard *et al.* (1989), studying gelatin gels, showed that the gel constituents were perfectly immobilized under a vitreous state for specimen depth around 5–10 μm. These conditions avoid, in particular, artifacts, such as water crystallization, that lead to a collapse, modification or demixing within the structure. Vitrification of pure water occurs on cooling to $T < -100$ °C at rates in excess of about 3×10^6 °C/s (Bald, 1986). A further consideration is that the gel slices prepared for the study by electron microscopy should not be disrupted when cutting a thin slice. In practice, these conditions are achieved for amylose when concentration is higher than 4% (w/w). Electron micrographs of two amylose concentrations (4·7 and 7·8%) corresponding to a depth of approximately 5 μm are shown in Fig. 1. The gel characteristics (mesh size, strand width) were determined from the observation of stereo images. It appears that amylose gels consist of networks of interconnected filaments in which distances between two cross-links are of the order of several hundreds of nanometres. As the orientation of the filaments is unknown, it is impossible to have a more accurate determination. For both concentrations studies, the strands consisted of a large amount of chains aggregated in a rod-like structure of 25 nm wide (sd 10 nm on 100 measurements). This indicates a mean strand width of 20 nm (sd 10 nm) after allowance has been made for the thickness of platinum-carbon coating (~5 nm). The strand width appeared independent of concentration as indicated by the similar average width of the filaments for the two gels studied.

Amylose gel structure was not destroyed by acid hydrolysis (HCl, 2·2N, 35 °C, 35 days), although the gel became opalescent and fragile. Due to this weakness, they cannot be characterized by freeze-etching. Hydrochloric acid erodes preferentially the amorphous fraction of starch (Robin *et al.*, 1975). The kinetics of acid hydrolysis of amylose gels (2·7–7·9% w/w) shown in Fig. 2, indicated a two-step process. In the first phase (5–10 days), amylose gels were relatively quickly hydrolyzed. The initial hydrolysis rate, ranging from 13 to 5% per day as the polymer concentration was increased from 2·7 to 7·9% w/w, was dependent upon the gel concentration. In the second phase, the rate of hydrolysis decreased and after 10 days became linear with a rate of 0·4 to 0·5% per day and was essentially independent of concentration. Extrapolation of the linear region to zero time allowed the determination of the easily degradable (F(%)) fraction. The resistant fraction (100−F(%)), assigned to the ordered fraction of the gel, increased from 67 to 82% as the amylose concentration increased from 2·7 to 7·9% (Table 1).

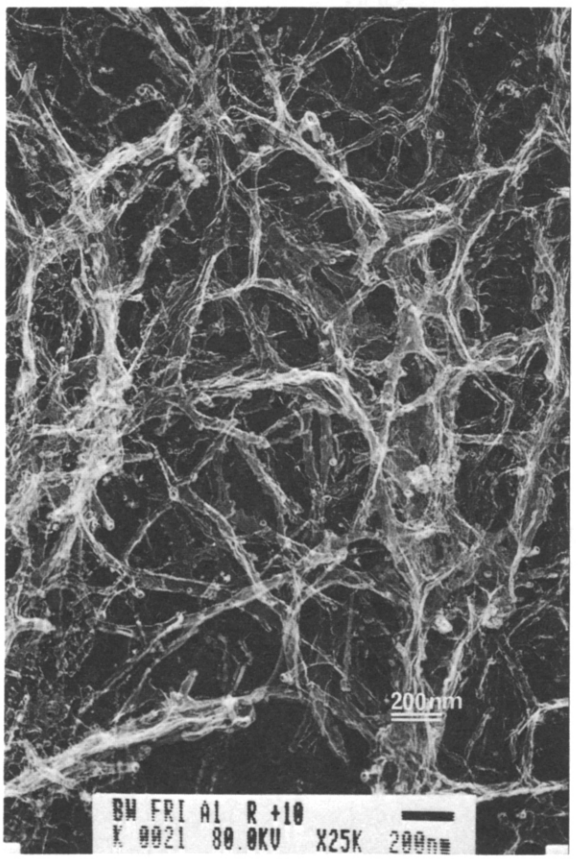
The *DP* and polydispersity of the residues obtained after 35 days of mild acid hydrolysis were determined. The chromatographic profile showed a single peak. The average degrees of polymerization DP_n and DP_w ranged from 26 to 31 and 56 to 73 respectively (Table 2). DP_n was independent of amylose concentration in the range studied, whereas DP_w increased significantly from 56 to 73 with gel concentration. Polydispersity expressed as the ratio DP_w/DP_n increased from 2·05 to 2·64 over the same range (Table 2). The characteristics



(a)



(b)



(c)

Fig. 1. Representative area of amylose gels prepared by the fast-freeze, deep-etch, rotary-shadowed replica method: (a) 4·7% amylose gel; (b), (c) 7·8% amylose gel.

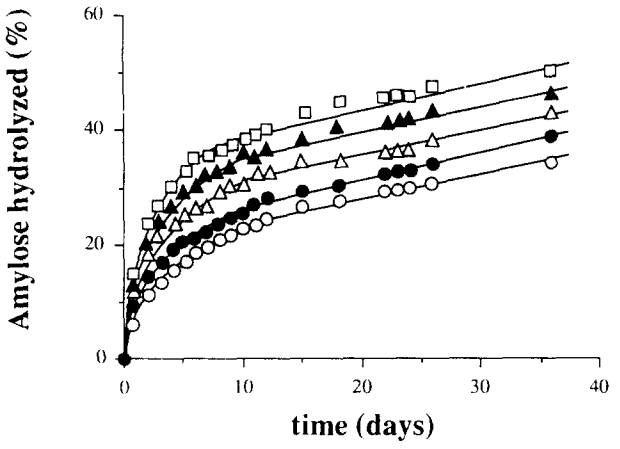


Fig. 2. Kinetics of acid hydrolysis of amylose gels of different concentrations: (□) 2·73%; (■) 3·64%; (△) 4·52%; (●) 5·12%; (○) 7·94%.

of the amylose gel residues (DP_n and DP_w) remained essentially the same after the inital hydrolysis (5 days). No fraction of intermediary degree of polymerization was observed in the early stage of the acid hydrolysis.

The thermal stability of the associated starch regions were also investigated. Native gels and gel residues from mild acid hydrolysis were studied by differential scanning calorimetry (DSC) over the temperature range 30–160 °C. At the concentrations examined (2·7–7·9%) native gels showed a broad endotherm between 95 and 135 °C with a peak maximum around 120 °C. The enthalpy change for the melting process, ΔH, calculated for the gels ranged from −8·5 to −8·9 J/g (d.b.) (Table 3). When these values were corrected for their effective amount of associated polysaccharide, as previously determined by acid hydrolysis, enthalpies ranged from −10·9 to −12·7 J/g (Table 3). These

Table 1. Easily degradable (F) and resistant ($100-F$) fractions of amylose gels of different concentrations determined by mild acid hydrolysis (HCl, 2·2N, 35 °C)

	Amylose concentration (%)							
	2·73	3·13	3·64	4·52	5·12	5·92	7·24	7·81
F(%)	33	33	31	26	24	22	19	18
$100-F$(%)	67	67	69	74	76	78	81	82

Table 2. Average degrees of polymerization (DP_n, DP_w) and polydispersity (DP_w/DP_n) of the residues of amylose gels obtained by mild acid hydrolysis

	Amylose concentration (%)							
	2·73	3·13	3·64	4·52	5·12	5·92	7·24	7·81
DP_n	28	26	31	26	29	29	27	32
DP_w	61	56	63	60	65	67	72	73
DP_w/DP_n	2·16	2·13	2·05	2·27	2·19	2·31	2·64	2·33

Table 3. Differential scanning calorimetry of native and acid treated amylose gels

	Amylose concentration (%)			
	2·73	4·52	5·43	7·94
Untreated gels				
T_i	98·8	108·7	101·6	95·0
T_{peak}	117·2	125·1	121·4	118·2
T_f	129·6	136·1	138·0	134·8
ΔH_{gel} (J/g)	−8·5	−8·5	−8·9	−8·9
$\Delta H_{crystalline}$ (J/g)	−12·7	−11·5	−11·7	−10·9
Acid treated gels				
T_i	108·5	113·0	117·8	112·8
T_{peak}	125·3	131·0	133·4	130·5
T_f	147·1	145·8	147·1	144·2
ΔH (J/g)	−14·2	−14·5	−14·6	−14·3

characteristics of the native gels appeared independent of the gel concentration, suggesting similar structures for the associated regions of the gel. The residues after mild acid hydrolysis gave sharper endothermic peaks, slightly shifted towards higher temperatures. The initial and final melting temperatures were 110 and 145 °C, respectively, with the peak centred around 130 °C. The melting enthalpies ranged from −14·2 to −15·5 J/g (d.b.) (Table 3). This corresponds to an increase of about 20%, and perhaps indicates that the mild acid hydrolysis is having a small effect on the organization of the associated regions.

DISCUSSION

Previous studies have suggested that amylose forms a gel as a result of phase separation, to produce a continuous polymer-rich phase (Miles *et al.*, 1984). A limited B-type crystallization of amylose chains occurs subsequently upon ageing. In these crystalline domains, amylose chains adopt double helical structures which aggregate into compact arrays (Imberty & Perez, 1988). The extent of organization of the polymer-rich phase is still a subject of debate (Miles *et al.*, 1984, 1985*a*, *b*; Gidley, 1989). No model of organization has yet related the three-dimensional microstructure of amylose gels to macromolecular organization.

The electron microscopy experiment shows, for the first time, the three-dimensional network of amylose gels. It indicates a structure of interconnected strands of about 20 nm wide, and must consist of assemblies of a large number of amylose chains. It suggests a fairly rigid structure resulting from this aggregation. The width of the filament is independent of amylose concentration. The increase in the density of filaments with amylose concentration is the main difference observed, leading thus to a reduced porosity for concentrated gels. The mesh size estimated from stereo images is of several hundred nanometres, although no statistical analysis of porosity was performed. A statistical determination of the gel porosity has been previously achieved by a size exclusion method (Leloup *et al.*, 1990). The mean pore size (R_p) ranged from 4 to 40 nm as the polymer concentration (C) decreased from 10 to 3% (w/w). The scaling relationship $R_p \sim C^{-\nu}$ with $\nu = 0{\cdot}82$ was obtained. For polymer solutions and gels, it has been demonstrated theoretically that the chain or filament conformation (rigid/flexible) affects the value of the exponent ν (De Gennes, 1979; Cukier, 1984; Phillies *et al.*, 1985, 1987; Phillies 1986). Thus, the predicted value of ν for a network of a rodlike polymer is 0·5 (Cukier, 1984), whereas ν lies in the range 0·5–1·0 for networks of flexible coils depending on the solvent/network interactions. The $C^{-0{\cdot}82}$ dependence of R_p upon polymer concentration, obtained for amylose gels (Leloup *et al.*, 1990), suggests that hydrodynamic behaviour of diffusing species is determined by a flexible component of the gel. This is an outstanding result, for a $C^{-0{\cdot}5}$ dependence would have been expected from the electron microscopy observations. Thus, the characteristics of the microstructure do not have the predicted effect and diffusive behaviour of probe species are not satisfactorily explained. This result could as well indicate that amylose gel is a filamentous structure defining a macroporosity (about several hundred nanometres) which entraps a network of microporosity (<40 nm) made of flexible amylose fragments.

The microscopy experiment gives no information on both the direction and the extent of organization of the amylose chains in the filaments. Mild acid hydrolysis demonstrates the presence of two fractions in the gel structure. An easily degradable fraction (F) accounting for 18–33%, corresponds to an unorganized amylose which is located directly on the structure surface (Robin *et al.*, 1975). The second fraction resistant to acid hydrolysis ranges from 67 to 82% of the gel, and is assigned to a partially crystalline fraction. It corresponds to the filamentous structure of the gels, as amylose gel is not destroyed by this chemical means. In a recent study, Cairns *et al.* (1990) showed for amylose gels which had reached a steady state, that the extent of crystallinity, as assessed by X-ray diffraction, was below 25% for a 10% concentration (w/w). However, the crystallinity of the amylose gel increased slightly (<10%) when hydrolyzed by α-amylases. A similar increase in crystallinity, or more accurately association, is observed by the DSC experiment. Amylose gels hydrolyzed by hydrochloric acid give sharper endothermic peaks and higher melting temperatures. This corresponds to an increase of ~20% in the enthalpy of melting. This phenomenon has also been observed with amylose-rich (Colonna *et al.*, 1982) or retrograded starches (Ring *et al.*, 1987; Mestres *et al.*, 1988*a*), assuming that the melting enthalpy is not dependent on crystallite size. This effect could be due to a partial recrystallization or reassociation of the dangling chains generated by the hydrolysis and located at the crystallite extremities (Kainuma & French, 1971, 1972). Crystallization of these short chains is a rapid process compared to the rate of acid hydolysis of amylose gels.

The chromatographic study of the gel residues obtained after acid hydrolysis allowed the determination of the average degrees of polymerization of the chains making the poorly crystalline associated fraction. These residues are partially organized under B-type crystallites. Imberty and Perez (1988) have stated that double helices of B-type crystalline structures are made of left-handed, six-fold helices repeating in 2·1 nm. From the average degrees of polymerization, this would imply that the crystalline residues are composed of an average eight turns of double helical structure. From the c-axis length (2·1 nm per turn), the size of the crystallite was evaluated to 10–25 nm, which is com-

parable with the filament width. Furthermore, the degrees of polymerization of the residues (26–73) allow the participation of a same chain in different crystallites. At this stage it may thus be proposed that amylose gels are made of three interconnected regions: amorphous, crystalline and intermediary zone.

The present results for amylose gels are comparable to a previous study (Jane & Robyt, 1984). Jane and Robyt studying retrograded amylose, obtained from dilute solutions (≤0·35% w/w), suggested a discontinuous model of organization. They stated that crystalline double helical regions, involved the participation of ~32D-glycosyl residues, expressed as the DP_n, and a length of ~10 nm. Crystalline regions were separated from each other by amorphous regions. Upon acid or enzymic treatments, the amorphous segments of amylose chains were hydrolyzed and crystallites 10 nm long were produced. Intermediary zones in contact with the crystallites crystallized after hydrolysis (Fig. 3). A similar mode for amylose gel structure may be proposed, consisting of a crystalline fraction embedded in an amorphous matrix. Average degrees of polymerization for each fraction may then be calculated for amylose as a function of concentration, from the knowledge of the easily degradable fraction (F) and the average degrees of polymerization DP_n and DP_w:

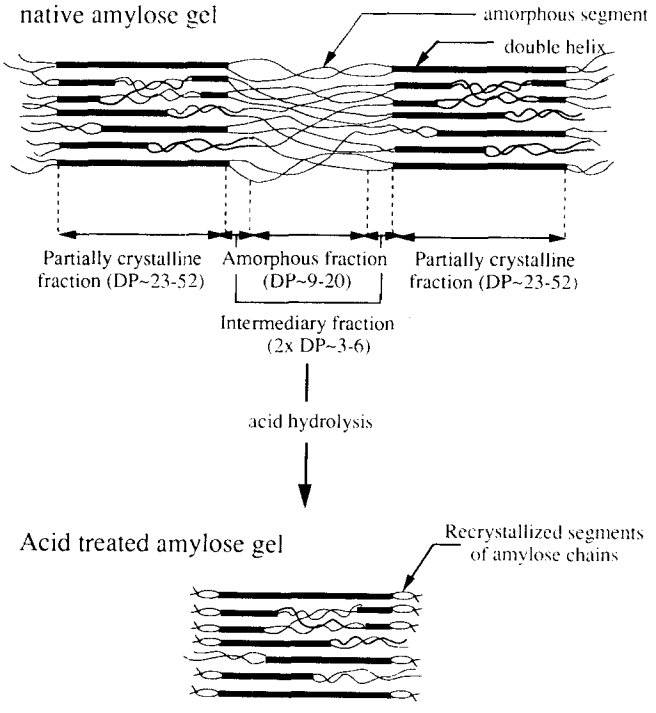


Fig. 3. Discontinuous model for amylose gels derived from Jane and Robyt (1984).

(1) The crystalline or associated fraction (C) represents an average of 80% of the acid hydrolysis residue, whereas the recrystallized fraction (R) accounts for the other 20% as demonstrated by DSC. Their average degrees of polymerization are evaluated as follows:

$$DP_n \times 0{\cdot}8 < C < DP_w \times 0{\cdot}8 \quad (6)$$

$$DP_n \times 0{\cdot}2 < R < DP_w \times 0{\cdot}2 \quad (7)$$

(2) The polymerization degree of the amorphous fraction (A) is calculated from the easily degradable fraction. Its average values are given by:

$$\frac{F}{100-F} \times DP_n < A < \frac{F}{100-F} \times DP_w \quad (8)$$

The calculated values, given in Table 4, indicate that the highest variations are found in the length of the amorphous segments of the amylose chains. Their average degrees of polymerization significantly decrease as the amylose concentration is increased.

In order to link microstructure and macromolecular organization, it is proposed that amylose chains aggregate in an infinite three-dimensional network. The chain segments inside the crystallites are disposed obliquely to the microfibre axis (Fig. 4). This model allows one to relate the microfibrous structure observed by electronic microscopy to macromolecular characteristics of the associated regions determined by acid hydrolysis. The average length (l) of the crystallite is as determined from the values presented in the Table 4 and crystallography data as follows:

$$\frac{DP_n \times 0{\cdot}8 \times 2{\cdot}1}{6} < l\,(\text{nm}) < \frac{DP_w \times 0{\cdot}8 \times 2{\cdot}1}{6} \quad (9)$$

Table 4. Characteristics of the crystalline, amorphous and intermediary fractions of the amylose gel formed at different concentrations

	Amylose concentration (%)							
	2·73	3·13	3·64	4·52	5·12	5·92	7·24	7·81
DP_n-DP_w	28–61	26–56	31–63	26–60	29–65	29–67	27–72	32–73
F(%)	33	33	31	26	24	22	19	18
Crystalline fraction (C)	22–49	21–45	25–50	21–48	23–52	23–54	22–58	26–58
Recrystallized fraction (R)	6–12	5–11	6–13	5–12	6–13	6–13	5–14	6–15
Amorphous fraction (A)	14–30	13–28	14–28	9–21	9–20	8–19	6–17	7–16

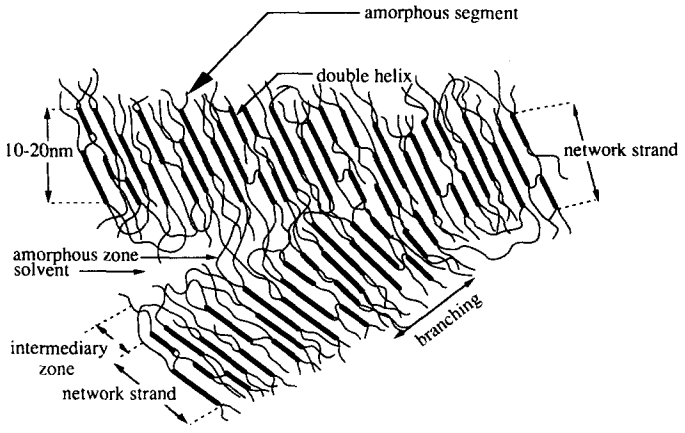


Fig. 4. Continuous model for amylose gels.

with 6 and 2·1 being respectively the number of glucosyl units and the *c*-axis length of an amylose helix. This length ranges from 8 to 18 nm, which is in good agreement with the filament width determined by electron microscopy (20 ± 10 nm). The aggregation of these segments generates a three-dimensional network. Therefore, a network strand would consist of contiguous associated blocks, aligned along the length axis of the mirofibre. Double helices would then be linked to others by loops of amorphous amylose segments, dangling in the gel pores. This fraction would be responsible for the hydrodynamic behaviour of the amylose gels. From steric consideration determined by the flexibility of α-glucan chains, the interconnecting loops should have a minimum of three glucosyl units which corresponds to a half α-cyclodextrin molecule. This condition is always fulfilled, even for the highest amylose concentrations studied. This model suggests the occurrence of both parallel and antiparallel packings, which correspond to two local energetic minima of amylose double helices (Imberty & Perez, 1988). This packing of crystalline blocks is easier to relate to the coil conformation of amylose chains in solution (Ring *et al.*, 1985).

These observations bring new arguments concerning the debate on gelation mechanism of amylose. Two theories have been proposed. Miles *et al.* (1985*b*) and Doublier & Choplin (1989) studying amylose gelation, observed initially an increase in turbidity ascribed to a phase separation process. Subsequently, an increase in elasticity was recorded, followed by, on a longer time scale, a crystallization process. In this gelation model, the two latter phases correspond to the establishment of the gel junction zones. In contrast, Clark *et al.* (1989) studying the gelation of fairly monodisperse amylose ($DP \sim 2500$) observed immediate increase in elasticity occurring before the turbidity development. They suggested that the formation of junction zones leads to a phase separation. In the present study, amylose gels had reached a steady state in terms of turbidity, elasticity and crystallinity developments. However, the model of organization could well agree with the first gelation mechanism. The phase separation would allow short range interactions between fragments of amylose. These could then adopt double helical structures which in a later phase aggregate and form a filamentous network. The authors' amylose gel model is not consistent with Clark's gelation mechanism. However, the gels studied by the present authors were at equilibrium. Therefore their structure, as proposed in this macromolecular model, cannot be used as a direct argument for one of these two theories.

These results need to be further substantiated. This proposed model could be tested using electron diffraction patterns of the network strands and could give information on the chain orientation within the associated blocks.

REFERENCES

Adkins, G.K. & Greenwood, G.T. (1966). *Starch/Stärke*, **18**, 213.
Bald, W.B. (1986). *J. Microscopy*, **143**, 89.
Björck, I., Nyman, M., Pedersen, B., Siljeström, M.N., Asp, G. & Eggum, B.O. (1986) *J. Cereal Sci.*, **4**, 1.
Björck, I., Nyman, M., Pedersen, B., Siljeström, M.N., Asp, G. & Eggum, B.O. (1987). *J. Cereal Sci.*, **6**, 159.
Bornet, F.R.J. *et al.* (1989). *Am. J. Clin. Nutr.*, **50**, 315.
Cairns, P., Leloup, V.M., Miles, M.J., Ring, S.G. & Morris, V.J. (1990). *J. Cereal Sci.*, **12**, 203.
Clark, A.H., Gidley, M.J., Richardson, R.K. & Ross-Murphy, S.B. (1989). *Macromolecules*, **22**, 346.
Colonna, P., Buleon, A., Lemarguer, M. & Mercier, C. (1982). *Carbohydr. Polymers*, **2**, 43.
Colonna, P., Barry, J.-L., Cloarec, D., Bornet, F., Gouilloud, S. & Galmiche, J.-P. (1990). *J. Cereal Sci.*, **11**, 59.
Cukier, R.I. (1984). *Macromolecules*, **17**, 252.
De Gennes, P.-G. (1979). In *Scaling Concept in Polymer Physics*. Cornell University Press, London, p. 45.
Doublier, J.-L. & Choplin, L. (1989). *Carbohydr. Res.*, **193**, 215.
Ellis, H.S. & Ring, S.G. (1985). *Carbohydr. Polymers*, **5**, 201.
Favard, P., Lechaire, J.-P., Maillard, M., Favard, N., Djabourov, M. & Leblond, J. (1989). *Biol. Cell*, **67**, 201.
Gidley, M.J. (1989). *Macromolecules*, **22**, 351.
Gidley, M.J. & Bulpin, P.V. (1989). *Macromolecules*, **22**, 341.
Heller, J., Pangburn, S.H. & Roskos, K.V. (1990). *Biomaterials*, **11**, 345.
Heuser, J. (1981). *Meth. Cell. Biol.*, **22**, 97.
Hizukuri, S., Takeda, Y., Yasuda, M. & Suzuki, A. (1981). *Carbohydr. Res.*, **94**, 205.
I'Anson, K.J., Miles, M.J., Morris, V.J. & Ring, S.G. (1988). *Carbohydr. Polymers*, **8**, 45.
Imberty, A. & Perez, S. (1988). *Biopolymers*, **27**, 1205.
Jane, J.-L. & Robyt, J.F. (1984). *Carbohydr. Res.*, **132**, 105.
Kainuma, K. & French, D. (1971). *Biopolymers*, **10**, 1673.
Kainuma, K. & French, D. (1972). *Biopolymers*, **11**, 2241.
Kalichevsky, M.T. & Ring, S.G. (1987). *Carbohydr. Res.*, **162**, 323.
Kalichevsky, M.T., Orford, P.D. & Ring, S.G. (1990). *Carbohydr. Res.*, **196**, 271.
Katz, J.R. (1930). *Z. Phys. Chem.*, **150**, 37.
Kitamura, S., Yoneda, S. & Kuge, T. (1984). *Carbohydr. Polymers*, **4**, 127.

Leloup, V.M., Colonna, P. & Ring, S.G. (1990). *Macromolecules*, **23**, 862.
Leloup, V.M., Colonna, P. & Buleon, A. (1991). *J. Cereal Sci.*, **13**, 1.
Levy, M.-C. & Andry, M.-C. (1990). *Int. J. Pharmaceutics*, **62**, 27.
McCann, M.C., Wells, B. & Roberts, K. (1990). *J. Cell Sci.*, **96**, 323.
Mestres, C., Colonna, P. & Buleon, A. (1988*a*). *J. Food Sci.*, **53**, 1809.
Mestres, C., Colonna, P. & Buleon, A. (1988*b*). *J. Cereal Sci.*, **7**, 123.
Miles, M.J., Morris, V.J. & Ring, S.G. (1984). *Carbohydr. Polymers*, **4**, 73.
Miles, M.J., Morris, V.J. & Ring, S.G. (1985*a*). *Carbohydr. Res.*, **135**, 257.
Miles, M.J., Morris, V.J., Orford, P.D. & Ring, S.G. (1985*b*). *Carbohydr. Res.*, **135**, 271.
Morris, V.J. (1990). *Trends in Food Science and Technology*, **1**, 2.
Orford, P.D., Ring, S.G., Carroll, V., Miles, M.J. & Morris, V.J. (1987). *J. Sci. Food Agric.*, **39**, 169.
Phillies, G.D.J. (1986). *Macromolecules*, **19**, 2367.
Phillies, G.D.J., Ullman, G.S., Ullman, K. & Lin, T.H. (1985). *J. Chem. Phys.*, **82**, 5242.
Phillies, G.D.J., Malone, C., Ullman, K., Ullman, G.S., Rollings, J. & Yu, L.P. (1987). *Macromolecules*, **20**, 2280.
Reed, J.P., Hall, F.R. & Trimnell, D. (1989). *Starch/Stärke*, **41**, 184.
Ring, S.G. (1985). *Starch/Stärke*, **37**, 80.
Ring, S.G. & Stainsby, S. (1985). *J. Sci. Food Agric.*, **36**, 607.
Ring, S.G., l'Anson, K.J. & Morris, V.J. (1985). *Macromolecules*, **18**, 182.
Ring, S.G. *et al.* (1987). *Carbohydr. Res.*, **162**, 277.
Robin, J.-P., Mercier, C., Duprat, F., Charbonniere, R. & Guilbot, A. (1975). *Stärke/Starch*, **27**, 36.
Swinkels, J.M. (1985). In *Starch Conversion Technology*, ed. G.M.A. Van Beynum & J.A. Roels. Marcel Dekker, New York, p. 15.
Tollier, M.T. & Robin, J.-P. (1979). *Ann. Technol. Agric.*, **28**, 1.
Treolar, L.R.G. (1975). In *The Physics of Rubber Elasticity*. Clarendon Press, Oxford, p. 72.
Trimnell, D. & Shasha, B.S. (1988). *J. Controlled Release*, **7**, 25.
Wing, R.E., Maiti, S. & Doane, W.M. (1987). *Starch/Stärke*, **39**, 422.
Wing, R.E., Maiti, S. & Doane, W.M. (1988). *J. Controlled Release*, **7**, 33.
Zobel, H.F. (1984). In *Starch Chemistry and Technology*, ed. R.L. Whistler, J.N. Bemiller & E.F. Paschall. Academic Press, New York, p. 285.