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Loss of crystalline and molecular order during starch gelatinisation: origin of the enthalpic transition*

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ABSTRACT

The disruption of molecular orders which occur during the gelatinisation of starch granules has been studied by isolating dried samples from maize, waxy maize, wheat, potato, and tapioca starches after defined thermal pre-treatments. Residual molecular and crystalline order was quantified by ^{13}C-c.p.-m.a.s.-n.m.r. spectroscopy and powder X-ray diffraction, respectively, and the results compared with residual gelatinisation enthalpy determined by d.s.c. For native starches, molecular (double-helical) order was significantly greater than crystalline order. Molecular and crystalline order were both found to correlate with the residual enthalpy of gelatinisation following thermal pre-treatment, indicating that both levels of structure are disrupted concurrently during gelatinisation. From the data obtained, predicted enthalpy values for the disruption of fully ordered and crystalline analogues of the starches studied were calculated, and compared with values for essentially fully ordered and crystalline model material. This comparison suggests that the enthalpy of gelatinisation primarily reflects the loss of molecular (double-helical) order.

INTRODUCTION

Gelatinisation is a term used to describe the irreversible changes which accompany the disruption of starch granule structure[1]. A recent attempt to define the process[2] started with the statement that "Starch gelatinisation is the collapse (disruption) of molecular orders within the starch granule...". As a result of this loss of order, swelling of starch granules is increased substantially and polysaccharide can be exuded. A combination of these features is considered to underlie the technologically important properties of pasting and retrogradation[2].

Molecular order within starch granules is often demonstrated by X-ray diffraction patterns corresponding to one of two limiting crystalline polymorphs (A, B) or the intermediate C form[3]. X-ray diffraction analyses of amylose fibres[4,5] and crystals[6] show that both polymorphs are composed of ordered arrays of double helices, most likely left-handed with parallel strands[6,7]. Polymorphic variation is centred on packing differences with only minor differences proposed in helix geometries[6,7]. In starch granules, amylopectin is considered to be responsible for crystallinity through ordered arrangements of double helices formed by adjacent branches within the structure[8,9]. Starch

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polymorphic form also affects high-resolution solid-state c.p.-m.a.s. ^{13}C-n.m.r. spectra[10,11] with signal patterns reflecting differences in unit-cell contents[10], most probably through helix-packing arrangements[11]. N.m.r. spectra of starch granules can be analysed in terms of a combination of amorphous and ordered components[11]. As n.m.r. spectroscopy is a short-distance range probe, it is considered that detected "order" corresponds to double-helix content in contrast to X-ray diffraction, which detects only those double helices that are packed in regular arrays. It is therefore not surprising that estimates of order by n.m.r. spectroscopy are considerably higher than those obtained from X-ray diffraction[11].

The most commonly used technique in studies of starch gelatinisation is probably differential scanning calorimetry (d.s.c.), which reveals an endothermic event at temperatures similar to those at which structural changes are observed. This event is often equated with the thermal melting of crystallites. Although observable X-ray diffraction patterns are lost during gelatinisation[12,13], there is no direct evidence that enthalpy changes (ΔH) observed by d.s.c. correspond quantitatively to melting of crystallites.

We now report on the loss of both crystalline (by X-ray diffraction) and molecular (by ^{13}C-n.m.r. spectroscopy) order for a range of starches as a function of gelatinisation extent, in order to probe any sequential loss of structural order and to investigate the structural origin(s) of the endothermic event.

RESULTS

For the five starch samples used in this study, Table I shows the results of solid-state n.m.r. spectroscopic and X-ray diffraction analyses of molecular and crystalline order, respectively, together with parameters of the endothermic transition monitored by d.s.c. The observed endotherms were found to be independent of starch concentration up to 25% w/w in water. Values obtained by all three techniques (Table I) are similar to those found in previous studies[1,8,11].

TABLE I

Structural and enthalpic properties of granular starches [a] and model crystallites

	Crystalline order[b] (%)	*Molecular order*[c] (%)	*Melting enthalpy (J/g)*	T_p (°)[d]
Wheat	20	39	9.7	57.7
Maize	27	43	14.3	70.2
Potato	24	40	16.2	58.3
Waxy maize	28	48	16.0	72.2
Tapioca	24	44	16.9	67.3
A-type debranched glycogen	>85[e]	>90[e]	35.0	76.9
B-type debranched glycogen	>85[e]	>90[e]	34.2	62.4

[a] After suspension in water at 25° followed by lyophilisation. [b] Determined by X-ray diffraction (±2%). [c] Determined by ^{13}C-n.m.r. (c.p.-m.a.s.) (±2%). [d] D.s.c. peak temperature. [e] Lower estimate due to lack of discernible amorphous features.

Highly crystalline material was obtained for both A- and B-type polymorphs from debranched glycogen[14]. ^{13}C-N.m.r.[11,15] and X-ray diffraction[14] data show very high levels of molecular and crystalline order, respectively. Accurate quantification of order is hindered by low levels of observable amorphous features (see Fig. 5 in ref. 15 and Fig. 4 in ref. 14), so estimated minimum values are shown in Table I. D.s.c. data suggest that crystalline debranched glycogens are useful models for ordered forms of granular starch, as transition temperatures are comparable (Table I). The effect of polymorphic form on transition temperature (Table I) is similar to that reported previously[16].

In order to obtain samples exhibiting degrees of structure loss, granular starches (5% w/w) were heated in water to various temperatures, cooled, and lyophilised. If heating was carried out to temperatures beyond the peak of the d.s.c. endotherm, then no residual order was detected following cooling and lyophilisation except for potato starch. A number of temperatures below the d.s.c. peak temperatures were therefore chosen for pre-treatment of each of the granular starches (except potato). Residual order and thermal behaviour were then characterised as for untreated starches. Table II shows temperatures accessed during heat/cool/dry treatments for each of the starches together with analyses of dried material expressed as a percentage of values found for unheated starches (Table I). Examples of the d.s.c., n.m.r., and X-ray diffraction results are shown in Figs. 1–3 for potato, wheat, and waxy maize starches, respectively.

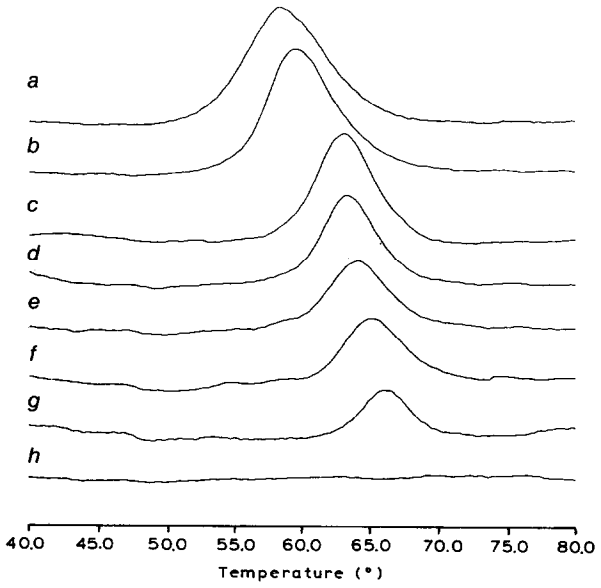

Fig. 1. D.s.c. traces showing gelatinisation endotherms for aqueous 20% potato starch heated at 10°/min after lyophilisation from aqueous suspensions treated at *a*, 25°; *b*, 57.4°; *c*, 60.4°; *d*, 60.9°; *e*, 61.5°; *f*, 62.2°; *g*, 63.3°; *h*, 67.0°.

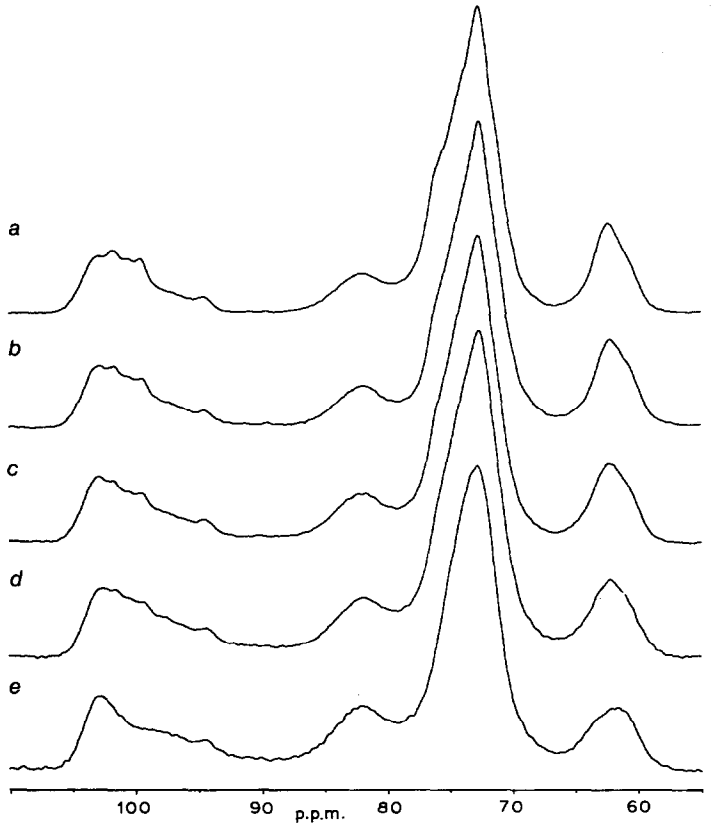

Fig. 2. C.p.-m.a.s. ^{13}C-n.m.r. spectra for wheat starch after pre-treatment at *a*, 25°; *b*, 53.2°; *c*, 53.8°; *d*, 54.6°; *e*, 59.0°.

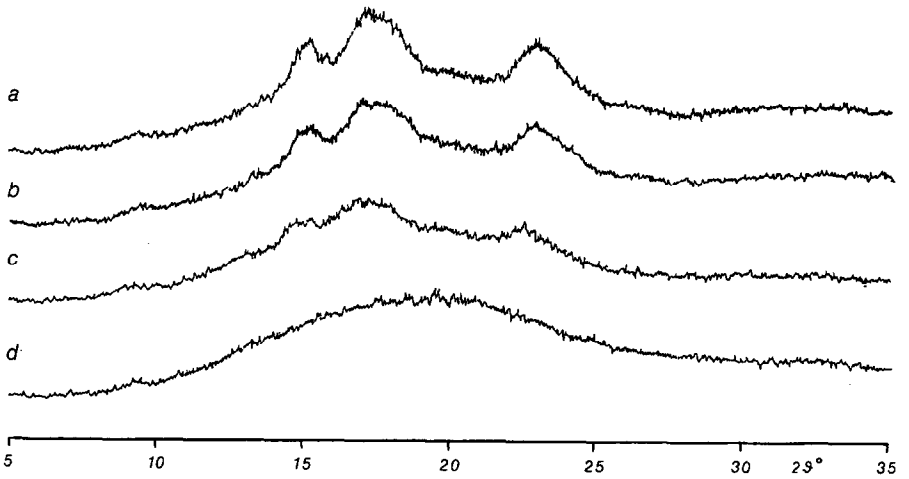

Fig. 3. X-ray powder diffraction patterns for waxy-maize starch after pre-treatment at *a*, 25°; *b*, 66.6°; *c*, 68.3°; *d*, 71.0°.

TABLE II

Relative loss of gelatinisation enthalpy and molecular and crystalline order for starches pre-treated by heating in an excess of water

Starch	*Pre-treatment temp.* (°)	*Crystallinity loss* [a] (%)	*Molecular order loss* [a] (%)	*Enthalpy loss* [a,b] (%)	T_p (°)[b]
Wheat	49.3	7	12	6	60.9
	53.2	18	30	34	63.2
	53.8	23	40	42	63.9
	54.2	35	49	66	64.8
	54.6	52	56	59	64.0
	55.6	56	64	65	64.8
	59.0	100	100	100	–
Maize	60.2	4	9	6	71.0
	64.7	15	19	29	71.8
	65.3	33	37	35	72.5
	65.8	30	35	36	73.0
	66.5	51	42	45	74.1
	67.5	67	53	62	75.0
	71.0	100	100	100	–
Potato	57.4	19	5	6	59.5
	60.4	54	37	30	63.1
	60.9	55	45	46	63.3
	61.5	74	50	55	64.1
	62.2	79	52	60	65.0
	63.3	90	58	75	66.0
	67.0	100	100	100	–
Waxy maize	64.5	3	14	14	73.9
	66.6	24	24	24	74.0
	67.2	32	26	37	75.8
	67.6	35	38	41	76.0
	68.3	51	41	50	76.5
	69.0	71	62	77	77.8
	71.0	100	100	100	–
Tapioca	61.1	14	7	16	69.9
	62.7	34	18	37	70.7
	63.0	33	18	29	70.0
	63.5	25	29	37	70.5
	64.0	52	41	58	70.8
	64.6	50	50	54	71.5
	69.5	100	100	100	–

[a] Expressed relative to values shown in Table I. [b] D.s.c. parameters observed on heating at 20% w/w in water.

DISCUSSION

Levels of molecular and crystalline order in native starches. — It is well established that starch granules contain regions of sufficient crystalline order to diffract X-rays. Powder diffraction patterns (*e.g.*, Fig. 3) show relatively broad peaks superimposed on an amorphous "halo". The relative intensity of these two features is used to estimate the level of crystalline order. Broad diffraction peaks indicate either imperfect or relatively small crystallites. N.m.r. spectroscopy is a molecular probe and is therefore considered to be sensitive to molecular order; *i.e.*, for starches, double-helix content. Estimates of the level of molecular order can be obtained through matching of amorphous/ordered composite spectra[11]. Comparison of levels of crystalline and molecular order (Table I and ref. 11) show that the former is significantly lower than the latter for all granular starches studied. This finding demonstrates that there are two types of double helices in starch granules, those involved in crystallites large/perfect enough to diffract X-rays and those which are not; together with substantial non-ordered material. This situation may be compared with that in amylose precipitates, which show broad powder X-ray diffraction patterns of variable intensity, but are essentially fully ordered at the double-helix level[17].

Isolation of starches after thermal pre-treatments. — There are two potential approaches to the study of starch gelatinisation events. One is to monitor the process as it occurs[13,18,19], and the second involves isolation and analysis of partially gelatinised samples. The advantage of the first approach is that it is direct; the disadvantage is that measurements using different techniques have to be made not only at the same temperature but also at the same heating rate, as kinetic considerations cannot be ignored. The advantage of analysing samples isolated at defined stages of the overall process is that a broad range of techniques can be applied to stable (dried) materials; the disadvantages are that structural changes may have occurred during the isolation process and that comparisons with direct monitoring of gelatinisation have to take into account any kinetic consequences of different thermal treatments.

As the purpose of this study was to monitor molecular and crystalline order by solid state methods, the isolation approach was used. Comparison of d.s.c. data in Tables I and II highlights differences in structure loss as monitored by the "direct" and "isolation" methods. Thus, for wheat, maize, and tapioca starches, pre-treatment to temperatures corresponding to the peak temperature (Table I) of the d.s.c. transition (*i.e.*, equivalent to ~ 50% completion of the transition) results in materials that exhibit less than an estimated 30% of original order and enthalpy levels (Table II). Waxy maize starch shows more extreme behaviour, with pre-treatment to a temperature (71.0°) lower than the d.s.c. peak temperature (72.2°) resulting in loss of detectable enthalpy as well as crystalline and molecular order (Table II). On the other hand, pre-treatment of potato starch to temperatures above the native d.s.c. peak temperature (58.3°) results in structure and enthalpy levels greater than 50% of original values (Table II), suggesting a degree of recrystallisation during the pre-treatment. The apparent structure loss observed for waxy maize starch was further investigated by d.s.c. experiments in which samples

were taken to temperatures similar to those used for isolation followed by cooling and reheating in the d.s.c. pan. The observed enthalpies on reheating were only slightly (~ 10%) lower than that fraction of the enthalpy for native material associated with temperatures higher than the pre-treatment temperature. Therefore, either kinetic effects of heating regimes or structure loss during drying are suggested to be the origin of differences between the d.s.c. and isolation data.

Taken together, our data suggest that, in general, isolation of starches after thermal pre-treatment does not accurately represent the state of starch at the pre-treatment temperature. Although greater correspondence might be achieved through careful control of heating rates and by ultra-rapid cooling from pre-treatment temperatures, data for waxy maize and potato starch show that not all starches have the same type of behaviour. Studies of gelatinisation events which rely on comparative measurements made during gelatinisation and on isolated materials[20] should be interpreted with caution.

Structure loss in thermally pre-treated starches. — Residual crystalline and molecular order have been estimated for five starches, each taken to seven different temperatures, and the results compared with residual d.s.c. enthalpy. In order to facilitate comparisons between the different techniques used, data are presented (Table II) as percentage decreases compared to controls (Table I) which were prepared by suspending starches in water at 25° followed by lyophilisation. Estimated errors in determining values for crystalline and molecular order are ± 2%. As typical values for controls (Table I) are 20–50%, then errors in estimating lower order levels, when expressed as loss percentages, are estimated to be ± 5–10% (Table II). At this level of discrimination, data presented in Table II show that, for each of the pre-treatment series (except potato starch), structure losses monitored by X-ray diffraction, ^{13}C-c.p.-m.a.s-n.m.r. spectroscopy and d.s.c. follow the same relative quantitative pattern. Data for potato starch may indicate (just significant) loss of crystalline order being in advance of loss of molecular order and enthalpy.

With the possible exception of potato starch, therefore, the present data suggest that crystalline and molecular order are lost concurrently during gelatinisation. Models for structure loss which invoke crystallite disruption followed, at a higher temperature, by helix melting[18,21] are not consistent with our observations. Conversely, there is also no evidence for the melting of non-crystalline double helices at a temperature lower than that of crystallite melting, suggesting that the thermal stability of double helices within granules is not affected significantly by the presence or absence of a crystalline environment. The observation of undetectably low levels of double helix (by n.m.r. spectroscopy) for thermally treated samples of each starch ("100% loss" in Table II) indicates that solubilisation and subsequent retrogradation of amylose is not significant for the particular treatment regime used.

Structural origin of d.s.c.-observed enthalpic event. — Table II shows that the endothermic enthalpy of gelatinisation of thermally pre-treated starches decreases to a comparable extent as both molecular and crystalline order. This finding clearly demonstrates that the endotherm reflects the melting of ordered structure for each of the

starches studied. Except for potato starch, both molecular and crystalline order are lost at a rate comparable to that of the loss of residual enthalpy with increasing pre-treatment temperature (Table II). In order to assess whether melting of crystalline or molecular (helical) order is the primary determinant of the endothermic enthalpy of gelatinisation, predicted enthalpies for melting of fully ordered starch-like systems have been calculated from the data in Table I. The data presented in Table III were obtained both by this single-point method and by graphical extrapolation to 100% molecular or crystalline order based on each of the data sets in Table II. These hypothetical enthalpies (Table III) can be compared with values for highly ordered model systems[14,22] of 35.4 ±1.8 (ref. 16), 35.0, and 34.2 J/g (Table I). Enthalpy values appear to be very similar for both A and B polymorphs, although there is ~ 15° between the peak temperatures[16] (Table I). From Table III, extrapolated enthalpies are very similar to those for essentially fully ordered model systems, provided the extrapolation is carried out to 100% molecular and not crystalline order. The validity of the debranched glycogen samples as models for the gelatinisation endotherm is reinforced by the fact that peak melting temperatures for native starches are within 10° of the appropriate polymorphic model (B-type for potato, A-type for the others), except for wheat starch which differs by ~ 20° from the model. It is interesting that extrapolation to 100% molecular order in wheat (Table III) gives poorer agreement with the model system than for the other starches examined. A possible explanation is that chain lengths associated with molecular order in wheat starch are shorter than for the other starches examined, and that this leads to lower values of both the enthalpy and temperature of melting. This inference would be consistent with the finding[23] that melting parameters of crystallised malto-oligosaccharides (prepared by fractionation of debranched glycogen[14]) show a marked chain-length dependence in the d.p. range 12–16 corresponding to probable helical lengths formed from amylopectin branches[24].

Data presented in Tables I–III therefore strongly suggest that d.s.c. endothermic enthalpy values primarily reflect loss of double-helical order rather than loss of crystalline register. This inference implies that the forces holding starch granules together are

TABLE III

Extrapolation of gelatinisation enthalpies to fully ordered systems based on analyses of molecular and crystalline order

Extrapolation end-point	*Extrapolated enthalpy values (J/g)*				
	Wheat	*Maize*	*Potato*	*Waxy maize*	*Tapioca*
100% Molecular order [a]	24.9	33.3	40.5	33.3	38.4
100% Crystalline order [a]	48.5	53.0	67.5	57.1	70.4
100% Molecular order [b]	25.2	33.6	38.8	33.0	34.2
100% Crystalline order [b]	44.8	54.4	68.5	53.6	66.0

[a] Extrapolated from data for granular starches shown in Table I. [b] Extrapolated using a least-squares fit of data for thermally treated starches represented in Table II.

largely at the double-helical level, and that the observed crystallinity may function as a means of achieving dense packing rather than as a primary provider of structural stability.

EXPERIMENTAL

Materials. — Starches used were standard commercial samples from ABR (wheat) and National Starch (all others). In order to obtain materials exhibiting various extents of structure loss, stirred aqueous suspensions of starch (5% w/v) were slowly heated to target temperatures over a period of 20–30 min (*i.e.*, ~ 1.5°.min^{-1}) followed by rapid cooling (cold running water) and lyophilisation. For comparison, each native starch sample was suspended in an excess of water at room temperature and lyophilised. All samples (10–12% H_2O) were stored at room temperature in sealed containers. No changes in d.s.c., X-ray diffraction, or ^{13}C-c-p.-m.a.s.-n.m.r. results were encountered after storage for several months. Glycogen was debranched using isoamylase and fractionated on Bio-Gel P-4 (− 400 mesh) as described previously[14]. Highly crystalline A- and B-type material was obtained from debranched glycogen after treatment of hot solutions at 40° (50% w/w) for A-type and 20° (10% w/w) for B-type[14]. Solidified material was collected, washed briefly with ice-cold water, and subjected to structural and d.s.c. analyses in the damp solid state (20–40% of water). X-ray powder diffraction and ^{13}C-c.p.-m.a.s.-n.m.r. results were essentially identical to those described previously[14,15].

Methods. — D.s.c. analyses were carried out with a Perkin–Elmer DSC 7 instrument operating at 10°/min, with data analysis performed using standard software. Powder X-ray diffraction patterns were obtained by using a Phillips powder diffractometer (PCW 1050/1390) mounted on a PW 1730/10 sealed-tube X-ray generator operating at the Cu-K_α wavelength (1.542 Å). To prevent any moisture-induced crystallinity changes, samples were analysed in a dry and sealed sample-holder unit. Powder patterns were analysed quantitatively by assessing the contribution of amorphous features (*e.g.*, Fig. 3d) to the total diffraction intensity over the angular range 5–30° 2Θ as described elsewhere[25,26]. This approach to obtaining amorphous/crystalline ratios for starches has been validated by quantitative comparison with results for celluloses[26,27]. ^{13}C-c.p.-m.a.s.-n.m.r. spectra were obtained with a Bruker MSL 300 instrument as described previously[11,15]. For quantitative analyses of ordered/amorphous ratios[11], spectra were compared with those generated by Fourier transformation of composite free-induction decays (f.i.d.'s) obtained by addition in various ratios of f.i.d.'s corresponding to fully ordered and amorphous starch samples (see Figs. 5 in refs. 11 and 15).

REFERENCES

1 H. F. Zobel, in R. L. Whistler, J. N. BeMiller, and E. F. Paschall (Eds.), *Starch: Chemistry and Technology*, 2nd edn., Academic Press, Orlando, 1984, pp. 285–309.
2 W. A. Atwell, L. F. Hood, D. R. Lineback, E. Varriano-Marston, and H. F. Zobel, *Cereal Foods World*, 33 (1988) 306–311.
3 A. Sarko and H.-C. H. Wu, *Staerke*, 30 (1978) 73–78.
4 H.-C. H. Wu and A. Sarko, *Carbohydr. Res.*, 61 (1978) 7–25.
5 H.-C. H. Wu and A. Sarko, *Carbohydr. Res.*, 61 (1978) 27–40.
6 A. Imberty, H. Chanzy, S. Pérez, A. Buleon, and V. Tran, *J. Mol. Biol.*, 201 (1988) 365–378.
7 A. Imberty and S. Pérez, *Biopolymers*, 27 (1988) 1205–1221.
8 D. French, in R. L. Whistler, J. N. BeMiller, and E. F. Paschall (Eds.), *Starch: Chemistry and Technology*, 2nd edn., Academic Press, Orlando, 1984, pp. 184–247.
9 D. J. Manners, *Carbohydr. Polym.*, 11 (1989) 87–112.
10 R. H. Marchessault, M. G. Taylor, C. A. Fyfe, and R. P. Veregin, *Carbohydr. Res.*, 144 (1985) c1–c5.
11 M. J. Gidley and S. M. Bociek, *J. Am. Chem. Soc.*, 107 (1985) 7040–7044.
12 J. Owusu-Ansah, F. R. van de Voort, and D. W. Stanley, *Cereal Chem.*, 59 (1982) 167–171.
13 H. F. Zobel, S. N. Young, and L. A. Rocca, *Cereal Chem.*, 65 (1988) 443–446.
14 M. J. Gidley and P. V. Bulpin, *Carbohydr. Res.*, 161 (1987) 291–300.
15 M. J. Gidley and S. M. Bociek, *J. Am. Chem. Soc.*, 110 (1988) 3820–3829.
16 M. A. Whittam, T. R. Noel, and S. G. Ring, *Int. J. Biol. Macromol.*, 12 (1990) 359–362.
17 M. J. Gidley, *Macromolecules*, 22 (1989) 351–358.
18 R. F. Tester and W. R. Morrison, *Cereal Chem.*, 67 (1990) 551–557.
19 M. J. Gidley and D. Cooke, *Biochem. Soc. Trans.* 19 (1991) 551–555.
20 H. Liu, J. Lelievre, and W. Ayoung-Chee, *Carbohydr. Res.*, 210 (1991) 79–87.
21 J. M. V. Blanshard, in T. Galliard (Ed.), *Starch Properties and Potential*, Wiley, Chichester, 1987, pp. 16–54.
22 S. F. Tanner, S. G. Ring, M. A. Whittam, and P. S. Belton, *Int. J. Biol. Macromol.*, 9 (1987) 219–224.
23 M. J. Gidley, unpublished observations.
24 S. G. Ring, P. Colonna, K. J. I'Anson, M. T. Kalichevsky, M. J. Miles, V. J. Morris, and P. D. Orford, *Carbohydr. Res.*, 162 (1987) 277–293.
25 P. Chinachoti and M. P. Steinberg, *J. Food Sci.*, 51 (1986) 997–1000, 1036.
26 S. Nara and T. Komiya, *Staerke*, 35 (1983) 407–410.
27 M. Khairy, S. Morsi, and C. Sterling, *Carbohydr. Res.*, 3 (1966) 97–101.