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Resistance to acid hydrolysis of lipid-complexed amylose and lipid-free amylose in lintnerised waxy and non-waxy barley starches

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ABSTRACT

Waxy barley starches (0.8–4.0% lipid-complexed amylose = L·AM, 0.9–3.4% lipid-free amylose = F·AM) and non-waxy barley starches (6.1–7.2% L·AM, 23.1–25.9% F·AM) were lintnerised by steeping in 2 M HCl at 35°C for 140 h. Material solubilised from the waxy starches was estimated to be 70.7% of their amylopectin (AP) plus 3.7% of their L·AM and F·AM, and material solubilised from the non-waxy starches was estimated to be 70.7% of their AP plus 28.9% of their L·AM and F·AM. The polysaccharide components of the insoluble residue were characterised by HPLC, GPC, and λ_{max} of the polyiodide complex. It was concluded that short chain-length (CL 16) material was from external chains of AP, intermediate material (modal CL 46) was from retrograded F·AM, and longer chain residues (CL 77, 120–130) were from lipid-complexed segments of L·AM. The starch lysophospholipids were completely hydrolysed to free fatty acids which remained complexed with L·AM residues. This was shown by the ^{13}C CP/MAS-NMR spectrum which had a clear resonance at 31 ppm from mid-chain methylene carbons of fatty acids in complexes. The C-1 signal of the L·AM residues also included a feature at 104 ppm indicative of single V_6 AM helices. The wide-angle X-ray diffraction patterns of the residues of non-waxy starches were Cc-type (= mixed A+B types), whereas the spectra of the original starches were A-type. It is suggested that, during the early stages of lintnerisation, amorphous F·AM was partially hydrolysed into material (CL < 120) that retrograded into double helices (with B-type crystallinity) that were resistant to hydrolysis. Evidence for some B-crystalline polymorph was also obtained from the ^{13}C CP/MAS-NMR spectra, which were consistent with a mixture of double helices and V-type glycosidic conformations, with only a small proportion of non-ordered regions. Broad DSC endotherms were found for both waxy (50–110°C) and non-waxy (50–110°C) lintner residues, which were assigned to disordering of double helices from short chains (modal CL 16) for waxy residues, together with disordering of longer chains (modal CL 46) in double-helix residues of F·AM and also V-helix residues of L·AM for non-waxy starch residues.

INTRODUCTION

Cereal starches are unusual in that they contain significant quantities of monoacyl lipids throughout the granules, in amounts closely related to amylose

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(AM)[1] or long-chain (1 → 4)-α-D-glucan content[2]. These lipids are lysophospholipids (LPL) in the *Triticeae* and mixtures of free fatty acids (FFA) and LPL in other cereal starches[1]. Since monoacyl lipids can form inclusion complexes with single-chain V_6 helices of AM, it has often been assumed that this is how these starch lipids occur in native granules, although the available evidence was equivocal[1,3]. We have now obtained proof from ^{13}C cross polarisation/magic angle spinning (CP/MAS)-NMR spectra and from DSC experiments that AM-lipid complexes exist in native barley starches[4], and we consider that, in these starches, AM occurs in two forms—as lipid-complexed amylose (L · AM) and as lipid-free amylose (F · AM). L · AM and F · AM are both amorphous and have different properties. Peak gelatinisation temperature (T_p) measured by DSC is increased by L · AM, but is lowered by F · AM[4]. Swelling at 80°C, above the gelatinisation temperature range, is strongly inhibited by L · AM, but F · AM appears to contribute to swelling[4]. Since gelatinisation and swelling are primarily properties of the amylopectin (AP) fraction, the results indicate that L · AM and F · AM must interact with AP physically during these events.

When starch granules are subjected to controlled acid hydrolysis (lintnerisation), amorphous material is considered to be hydrolysed much faster than the crystalline domains[5], which comprise ordered double helices formed by external A and B_1 chains of AP (nomenclature according to Hizukuri[6]); AM, which is part of the amorphous material, *appears* to be hydrolysed quite rapidly[5,7–10]. When we measured soluble α-D-glucan from lintnerised barley starches covering a wide range of AM and lipid contents, we found that yields were much lower than expected, and this led to the discovery that L · AM and F · AM are both resistant to acid hydrolysis. These studies are described in this paper.

EXPERIMENTAL

Materials.—Starches isolated from eleven waxy barleys and six non-waxy barleys for studies of AM–lipid relationships, gelatinisation, and swelling properties[4,11] were used.

Lintnerising.—Starches were lintnerised by steeping in 2 M HCl at 35°C for 140 h, as described previously[12,13]. Soluble α-D-glucan was quantified as glucose, using an enzymic method[14], and multiplied by 0.9 to obtain the weight of original α-D-glucan that had been hydrolysed.

Chemical analyses.—A colorimetric method was used to determine apparent AM and total AM content of starches (native lipids present and absent, respectively)[15]. Native and lintnerised starches were debranched with isoamylase and fractionated by gel-permeation chromatography (GPC) on a column packed with Sephacryl S-200 (Pharmacia), using continuous enzymic measurement of α-D-glucan to monitor the effluent[16]. Alternatively, the debranched starches were fractionated by high-performance liquid chromatography (HPLC) with refractive index detection[13]. GPC and HPLC columns were calibrated with linear (1 → 4)-α-D-glucans

synthesised using potato phosphorylase[13]. The chain length (CL) of linear α-D-glucans was also calculated from λ_{max} (nm) of the polyiodide complex prepared under standard conditions[15], using the relationship

$$CL = 3290/(635 - \lambda_{max})$$

derived by Morrison and Karkalas from published data[17]. This relationship is useful for CL = 35 to 100, but is not accurate for CL > 110.

Physical measurements.—^{13}C CP/MAS-NMR spectra and wide-angle X-ray diffraction spectra were obtained as before[18–20]. DSC was used to determine the dissociation temperature of L · AM complexes in native and lintnerised starches[4,12]. Samples were also examined using a microscope fitted with a Kofler hot-stage.

RESULTS AND DISCUSSION

Starch composition and changes on lintnerising.—The composition of the eleven waxy and six non-waxy starches, and the amounts of starch hydrolysed after lintnerising for 140 h, are given in Table I. Previous experiments with waxy rice starches[12], which were 100% AP, established that the material hydrolysed should include all of the amorphous parts and a little of the crystalline parts of the AP, leaving a residue with a chain length similar to the external chain lengths in the original AP. This was verified with mature Waxy Hector and Hector barley

TABLE I

Composition of eleven waxy and six non-waxy barley starches[4,11] and amounts of starch hydrolysed after lintnerisation for 140 h

Starch	AP (%)	L·AM (%)	F·AM (%)	Starch hydrolysed (%)
Waxy				
Summire Mochi	98.3	0.8	0.9	70.6
Dango Mugi	97.9	1.2	0.9	70.3
Masan Naked	97.6	1.5	0.9	70.1
Tokushima Mochimugi, I	96.9	1.7	1.4	69.6
Chalibori	96.6	1.6	1.8	68.2
Tokushima Mochimugi, II	96.4	2.1	1.5	69.9
Iyatomi Mochi	96.1	2.1	1.8	68.8
Bozu Mochi	94.6	3.2	2.2	70.3
Wapana	93.5	3.4	3.1	66.4
Wanupana	93.5	3.5	3.0	66.7
Washonupana	92.6	4.0	3.4	66.7
Non-waxy				
Chalky Glen	70.8	6.1	23.1	61.6
Midas	69.8	6.2	24.0	59.2
Hector	69.6	5.4	25.0	58.6
Shopana	69.5	7.0	23.5	55.6
Compana	69.5	7.2	23.3	54.2
Glen	67.3	6.8	25.9	58.4

starches. L · AM and F · AM are both amorphous[4] and, according to the literature[5,7–10], much of the AM is hydrolysed over the first 3–5 days of lintnerising, so the material hydrolysed over 140 h should have included most of the L · AM and F · AM. The results presented here show that these assumptions about L · AM and F · AM were not correct.

For the waxy starches, the amount of starch hydrolysed was inversely correlated with AM content ($r = -0.829$, $P < 0.001$) according to the equation

$$\text{Waxy starch hydrolysed (\%)} = 70.7 - 0.671\ [\text{L} \cdot \text{AM} + \text{F} \cdot \text{AM (\%)}] \qquad (1)$$

The amount of starch hydrolysed and the residue from a starch that is pure AP were thus 70.7 and 29.3%, respectively.

If there was no strong interaction between AP and L · AM or F · AM in the native starch granules, they should have been hydrolysed independently; i.e., in a starch containing 90% AP, there should have been 0.707 × 90% hydrolysed starch derived from AP, and the remainder must come from L · AM and F · AM. Using this approach, eq. *1* can be rewritten in the form

$$\text{Waxy starch hydrolysed (\%)} = 0.707\ \text{AP (\%)} + 0.037\ [\text{L} \cdot \text{AM} + \text{F} \cdot \text{AM (\%)}] \qquad (2)$$

and this infers that only 3.7% of the [L · AM + F · AM] was hydrolysed. If L · AM and F · AM had been extensively hydrolysed, as the literature suggested (above), the constant 0.037 would have been nearer 1.0; hence, the original assumption was clearly wrong.

The data for the six non-waxy starches were too scattered to give good regression analyses, so mean values (30.6% original L · AM + F · AM, 57.9% starch hydrolysed) were used to obtain the relationship

$$\text{Non-waxy starch hydrolysed (\%)} = 0.707\ \text{AP(\%)} + 0.289[\text{L} \cdot \text{AM} + \text{F} \cdot \text{AM(\%)}] \qquad (3)$$

This infers that 28.9% of the [L · AM + F · AM] was hydrolysed, compared with only 3.7% in the waxy starches. These interpretations were supported by analyses of the lintner residues described below.

Composition of lintnerised residues.—HPLC of debranched native starches gave chromatograms with a peak near the void volume (CL > 600) and a trimodal profile of short chains (CL = 46–48, 23 and 15) from AP, as described before[11,13]. The debranched lintner residues also gave a small peak near the void volume, which was probably a retrogradation artifact since it was not obtained when the samples were fractionated at pH 9–10 by gel-permeation chromatography[13,16]. The principal peak in the GPC chromatogram from the lintner residue of waxy barley starch was CL 16–17 (Fig. 1A), similar to that reported for amylodextrins from potato[7], maize[8,10], and rice[9,21] starches. Debranching did not alter the profile significantly, and caused only small changes in λ_{max}, from which it was concluded

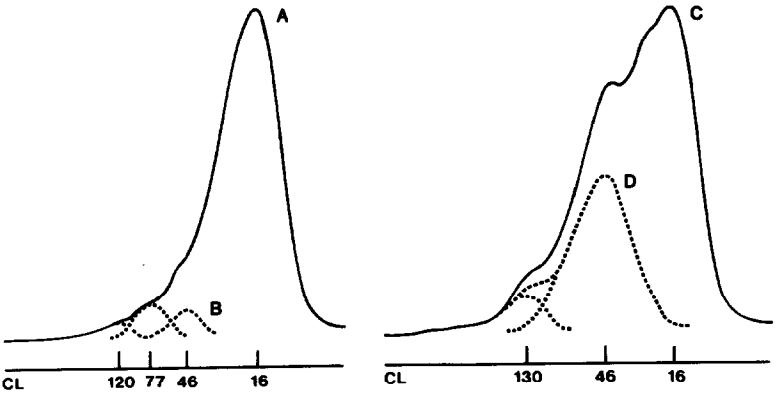

Fig. 1. GPC elution profiles of lintner residues from waxy starch (Bozu Mochi, left) and non-waxy starch (Chalky Glen, right). A, Full waxy profile; B, three minor components (CL 120, 77, and 46) giving shoulders to profile A if main peak of CL 16 is nearly symmetrical; C, full non-waxy profile with main peak of CL 16 drawn to same scale as A; D, profile C minus profile A, to give sub-profile of residues from L·AM and F·AM, which are interpreted as consisting of two components (CL 130 and 46).

that few (1 → 6)-α-D-glucosidic bonds survived lintnerisation for 140 h, in agreement with other reports[5].

Since the CL 16 peak in the lintner residue was almost the same length as the external A-chains in AP (indicated by the CL 15 peak in the HPLC chromatogram of debranched native starch), it was considered to be the lintner residue of AP. The long B_2, B_3, and B_4 chains of AP, which span two, three, or four of the smallest crystallisable domains, respectively[6], do not survive extensive lintnerisation[12], and were presumably cleaved in the vicinity of α-(1 → 6) branch points where the structure should be amorphous. Therefore, the minor components that eluted earlier were interpreted as three peaks of CL 120, 77, and 46 (Fig. 1B) derived from AM, assuming that the major peak (A) had near-Gaussian symmetry.

The elution profile of the lintner residue of the non-waxy starch also had a major peak of CL 16 (Fig. 1C, drawn to the same scale as peak A). Profile D was obtained by subtracting A from C to reveal the additional residues from F · AM and L · AM. Since ~ 71% of the [L · AM + F · AM] survived lintnerising (eq. *3*) and the original starches contained 24.1% F · AM (= 79% of total AM), it would be reasonable to ascribe the broad CL 46 peak to residues of F · AM and the minor narrower peaks of CL 77 and 120–130 to residues of L · AM. When pure AM (i.e., F · AM) was retrograded and then lintnerised under the same conditions, it gave a single peak of CL 50 with considerable polydispersity. Similar results with retrograded AM have been reported before[22,23]. Complexes of AM with C18:0 fatty acid (i.e., L · AM) gave narrow peaks of CL 122, 91, and 45, while C20:0 fatty acid complexes hydrolysed for various times gave peaks of CL 166–181, 96–105, and 62 (Karkalas and Morrison, unpublished results). Although they did not correspond exactly to the peaks in Figs. 1B and 1D, they do support the conclusion

that the CL 120–130 and 77 components, and perhaps a little of the CL 46 components, were residues of L · AM–lipid complexes.

The proportions of components in the lintner residue from Bozu Mochi were estimated from Fig. 1 (A and B) peak areas to be 91% from AP, 3% from F · AM, and 6% from L · AM; this compares reasonably well with predicted values (using eq *2* and Table I) of 85, 6, and 9%, respectively. Similarly, the residue from Chalky Glen was estimated to be 63% from AP, 28% from F · AM, and 9% from L · AM; the comparable predicted values (using eq *3* and Table I) were 50, 40, and 10%, respectively. Similar estimates were obtained by colorimetric analyses of the residues from two other waxy and two non-waxy starches (below).

The compositions of the starches determined by colorimetric analysis are given in Table II (top section), together with predicted values for the lintner residues. It was assumed that the amylose residues ("AM") had an average CL of 50; hence, iodine binding capacity would have been 57% of that for normal AM[24]. Apparent "AM" values, determined on residues with lipid present, would correspond to F · "AM", and the increase in "AM" content observed after removing lipid, Δ"AM", would correspond to L · "AM" residues[15]. Of the waxy starches, one-third survived lintnerisation as a residue with a high "AM" content, and there was sufficient lipid to give Δ"AM" values greater than for the original starch, although less than predicted. In the two non-waxy starches, total "AM" contents were very close to predicted values, but "AM" values were less than half.

Estimates of chain lengths were made from λ_{max}. Taking a mean value of 564 nm for apparent "AM" (= F · "AM"), CL was estimated to be 46, which agrees

TABLE II

Predicted and experimental (colorimetric analysis) values for the composition of the lintner residues from two waxy and two non-waxy barley starches

Property	Waxy		Non-waxy	
	Dangomugi	Washonupana	Midas	Hector
Original starch, experimental [a]				
ΔAM (%)	1.2	3.6	6.4	6.1
L·AM (%)	1.2	4.0	6.2	5.4
F·AM (%)	0.9	3.4	24.0	25.0
Lintner residue (%)	29.7	33.3	40.8	41.4
Lintner residue, predicted [b]				
L·"AM" (%)	3.7	11.3	12.1	10.5
F·"AM" (%)	2.7	9.5	46.6	48.5
Total "AM" (%)	6.4	20.8	58.7	59.0
Lintner residue, experimental				
λ_{max} apparent "AM" (nm)	555	563	566	562
λ_{max} total "AM" (nm)	571	579	571	569
Δ "AM" (%)	2	5	4	5
Total "AM" (%)	18	28	59	57

[a] From Table I. [b] Using eq *2* for waxy residues, and *3* for non-waxy residues.

well with the HPLC and GPC values. The presence of longer chains from L·"AM" in the total "AM" increased λ_{max} by 16 nm in the waxy starches, which had less F·"AM" than the non-waxy starches, but this probably underestimated the CL of L·"AM" since a λ_{max} of 564 + 16 = 580 nm corresponds to CL 60, which is much less than was found by HPLC and GPC.

In all four starches, ca. 80% of the lipids (measured as FAME) were recovered in the residues, but phosphorus content (a measure of LPL content) was nil, as noted before[9]. Thin-layer chromatography of the solvent-extracted lipid[25] revealed only FFA, with no traces of monoglycerides or LPL, which shows that the LPL were completely hydrolysed during lintnerisation, with loss of water-soluble glycerophosphate or glycerol and phosphate esters, leaving FFA complexed with "AM" and probably some uncomplexed FFA from the small fraction of L·AM that was completely hydrolysed (see eqs *2* and *3*).

Nature of the acid-resistant residues.—The acid-resistant parts of AP are considered to be the most crystalline ordered regions, consisting basically of double helices of external chains, and it would be reasonable to suppose that the resistant forms of "AM" were also crystalline or ordered in a similar manner, even though the original L·AM and F·AM were amorphous[4]. In the case of L·AM, the lipid-complexed segments of single-chain V_6-AM helices appear to be resistant to hydrolysis. They could account for low yields of soluble oligosaccharides found after hydrolysis of non-waxy starches[5,26], and explain the insoluble particles and AM-lipid complexes that have been reported in acid and enzymic hydrolysates of cereal starches[27–30]. Retrogradation during the acid hydrolysis of solutions of pure AM has also been suggested as a cause of low yields of soluble sugars[31]; this could occur if fragments (CL < 120) released during the early stages of hydrolysis retrograded rapidly into the very insoluble double-helix form[32,33]. Those concepts were tested by physical tests on the lintner residues from the waxy and non-waxy starches described in Table II.

The ^{13}C CP/MAS-NMR spectrum of native non-waxy barley starch had a weak resonance at 31.2 ± 0.4 ppm, corresponding to 0.3 mol% (theoretical, 0.4 mol%) mid-chain methylene carbons of the FA of LPL in V_6 AM complexes[4]. The FA in the LPL are 50% unsaturated; hence, the free lipid (not complexed with AM), and FFA derived from it, are liquid at ambient temperature and they would not give any signal under the CP/MAS conditions used here[4]. In the lintner residue, there was a stronger resonance at 31.4 ppm (Fig. 2), showing that the FFA (from the acid-hydrolysed LPL) were still complexed with "AM". The signal strength from the lintner residue was 3.3 times stronger than that from the native starch, and slightly greater than the enhancement predicted from eq *3* and Table II. The NMR spectra also showed increased resolution of the feature at 103–104 ppm (Fig. 3), characteristic of glucosyl C-1 in V_6 AM-lipid complexes[19,34,35], especially in the non-waxy starches.

Since the analytical data (above) were consistent with hydrolysis of 29% of the L·AM (eq *3*), and only one-seventh (14%) of the AM in L·AM complexes is

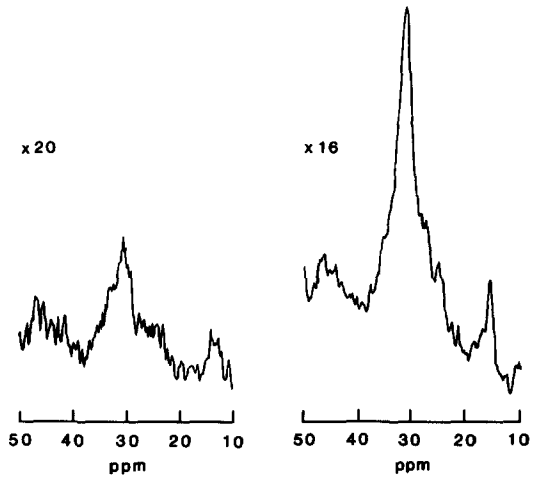

Fig. 2. ^{13}C CP/MAS-NMR spectra (with indicated scale expansion relative to Fig. 3) at 25 MHz over the region 10–50 ppm, showing resonance at 31 ppm of mid-chain methylene carbons of fatty acids in V_6 AM complexes in native Chalky Glen starch (left) and the lintner residue (right).

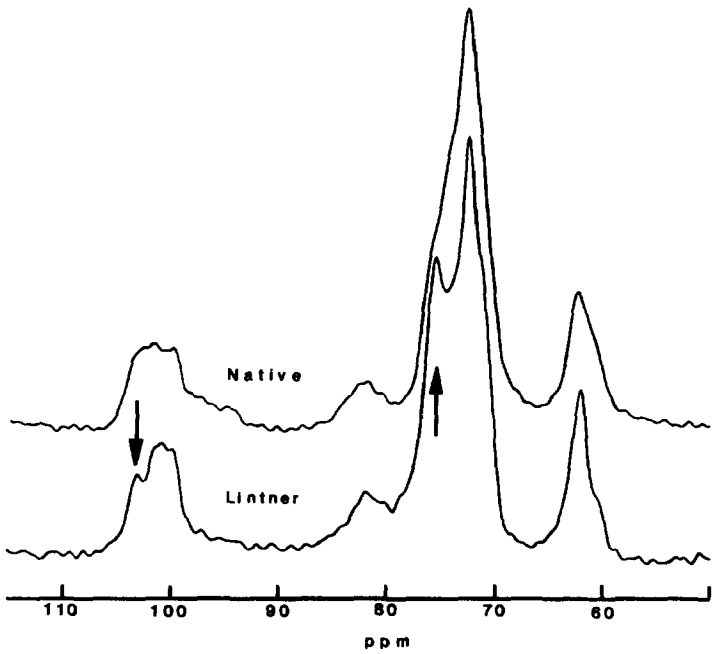

Fig. 3. ^{13}C CP/MAS-NMR spectra of native Midas starch (top) and its lintner residue (bottom) over the range 50–110 ppm. Arrows indicate features at 76 and 103–104 ppm discussed in the text.

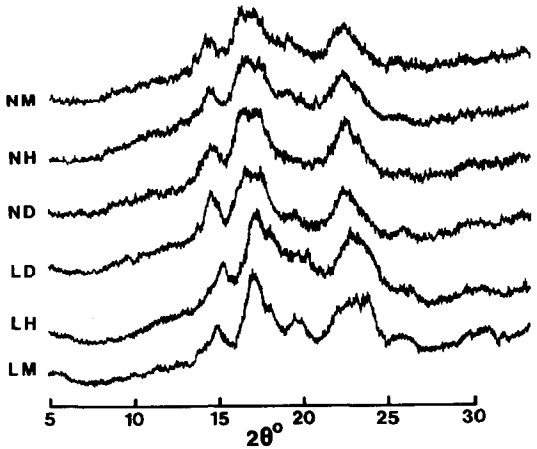

Fig. 4. Wide-angle X-ray diffraction spectra of native starches (N) from Midas (M), Hector (H), and Dangomugi (D) barleys, and their lintner residues (L).

non-helical (between lipid-complexed segments)[36,37], a small amount of L · AM must have been completely hydrolysed to soluble oligosaccharides with liberation of free lipid. This was presumably lost in the process of recovering the lintner residues, since only 80% of the original lipid was recovered in the residue. However, as noted above, if there had been any free lipid in the lintner residue, it would not have been detected by CP/MAS-NMR

Waxy and normal cereal starches give an A-type X-ray diffraction pattern from the ordered regions of AP, and a B-type pattern is only obtained when the starches have been gelatinised and the AP allowed to retrograde slowly[38,39]. The B-type pattern is also given by retrograded AM[32,38,40]. Fig. 4 shows that the native starches all gave normal A-type patterns. Dangomugi (97.9% AP) gave a lintner residue that had an identical pattern, showing that the type of crystallinity in AP was not altered by lintnerisation. However, the lintner residues of the two non-waxy starches, Midas and Hector, gave a Cc-pattern (Fig. 4, LM and LH) that comprised a mixture of A- and B-type patterns[41]. This is consistent with an A-pattern from the AP residues and a B-pattern from the retrograded F · "AM" residues.

The V-pattern characteristic of crystalline AM-lipid complexes is not given by amorphous complexes[35] or (normally) by complexes in native cereal starches[38,39]. There was no detectable V-pattern in the spectra of the lintner residues, perhaps because the L · "AM" residue was still amorphous, or (if it had been crystalline) because there was too little (Table II) to give resolvable spectral features. In another experiment (Tester, Morrison, Karkalas, and Gidley, unpublished results), all crystallinity in a sample of wheat starch was destroyed by prolonged ball-milling and all AP was degraded to low molecular weight fragments, but L · AM and F · AM were not affected. When this starch was lintnerised, the residue, which was exclusively from L · AM and F · AM, gave an X-ray diffraction pattern that was a

mixture of roughly equal parts of V-pattern from L · AM residues and B-pattern from F · AM residues. The ^{13}C CP/MAS-NMR spectrum of this residue confirmed that there was a substantial amount of V-helix AM, and lipid complexed with "AM", as in the present study.

Major changes in ^{13}C CP/MAS-NMR polysaccharide spectra were observed following lintnerisation (Fig. 3). In the C-1 region (90–110 ppm), intensity was significantly greater in the range 99–102 ppm (characteristic of double helices[19]) and less in the range 93–99 ppm (part of the signal envelope for non-ordered material[19]). A signal at 103–104 ppm, characteristic of V-type glycosidic conformations[19,34,35], was more resolved in the lintner residues and was of greater intensity than in the native starches, except for Midas.

These results suggest that double-helix content was enhanced relative to non-ordered ("amorphous") conformations following lintnerisation, and that a significant fraction of the non-double-helical material had V-type glycosidic conformations. Such conformations were not due solely to residues of L · AM, as they were observed with approximately equal abundance for both waxy and non-waxy starches. To explain the V-conformation for the waxy residues, it is proposed that one factor causing acid resistance is hydrogen bonding from HO-3′ to HO-2 of consecutive glucosyl residues, characteristic of V-type structures in solution[42] and in solid[43] states. This would also be a significant factor in the acid resistance of L · AM.

Other features of the ^{13}C CP/MAS-NMR spectra consistent with the above analysis included an increase in resolution of a signal at 82 ppm, assigned to C-4 of V-type conformations, and a sharpening of features in the C-2,3,4,5 region (70–79 ppm). For non-waxy starches (Fig. 3), a pronounced signal near 76 ppm was assigned to B-type double helices from crystalline residues of F · AM, as indicated by X-ray diffraction spectra.

Estimates of crystallinity and double-helix content were made from the X-ray and NMR spectra (Table III). Theoretically, if starch (60–70%) that was mostly amorphous had been removed as solubles, the lintner residue should have been mostly crystalline, but this was not so. Similar observations have been made before, but not explained[5], although another report[44] indicates a substantial increase in crystallinity of potato starch following similar lintnerisation treatments. As discussed above, it is possible that acid-resistance could be due to V-type local conformations as well as double helices.

Dangomugi was more crystalline than the two non-waxy starches but, surprisingly, Washonupana was not. This pattern was also found in the lintner residues, except that Midas residue was appreciably more crystalline than the original starch (as it should have been, in theory) and the other residues. Midas lintner residue also showed the greatest change in the ^{13}C CP/MAS-NMR spectrum at 76 ppm, attributed to B-type crystalline F · "AM" residues, and evidently attained greater long-range order than the other lintnerised starches.

Double-helix content, from external chains of AP, was greater in the waxy starches than in the non-waxy starches (Table III), reflecting their higher AP

TABLE III

Relative crystallinity (from X-ray spectra) and double-helix contents (from ^{13}C CP/MAS-NMR spectra) of two waxy and two non-waxy barley starches and their lintner residues

Property	Waxy		Non-waxy	
	Dangomugi	Washonupana	Midas	Hector
Crystallinity (%)				
Native starch	45	34	34	36
Lintner residue	42	33	54	39
Double helix (%)				
Native starch	55	50	40	44
Lintner residue	57	52	66	55

contents. After lintnerisation, double-helix contents in the lintner residues from the waxy starches were little changed, but the non-waxy starches (notably Midas) had appreciably higher double-helix contents which are attributed to retrograded F · "AM". The fact that the double-helix content of the lintner residues of the waxy starches was not greater than for the native starches (cf. crystallinity) reflects the overall transformation from a double-helix/non-ordered composite to a predominantly double-helix/V-type-conformation composite following lintnerisation.

Differential scanning calorimetry.—Since the lintner residues retained remnants of AP, L · AM, and F · AM with various levels of order and structure, it was of interest to study their behaviour on heating in water, compared with the gelatinisation of the native starches which gave normal DSC thermograms[4].

Microscopic observation showed that the residues were not birefringent and did not swell significantly at ambient temperature, but disintegrated over a broad temperature range when heated. This behaviour was mirrored in DSC thermograms (Fig. 5), which showed broad endothermic features for both waxy and non-waxy lintner residues. Data for waxy residues were similar to those reported recently[45] for a range of waxy starches, but data for non-waxy residues differed significantly from published data[44] for wheat and potato starch residues which showed features only up to 85°C.

DSC data for native starches were highly reproducible, but thermograms for lintner residues were not always superimposable although the broad monomodal and biomodal features illustrated in Fig. 5 were always observed for waxy and non-waxy starches, respectively. It was also found that the thermograms for lintner residues were less smooth than for native starches at the same instrument sensitivity. It is possible that this was due to convection within the DSC pan caused by irregular dissolution of residue particles, whereas swelling of granular starches was more controlled in terms of thermal motion. Enthalpy values for lintner residues were also less reproducible than for granular starches, possibly due to heat flow artifacts attributed (above) to convection caused by dissolution. Values obtained were 17–19 J/g for waxy lintner residues and 20–25 J/g for non-waxy lintner residues. These values were substantially greater than for the original waxy and

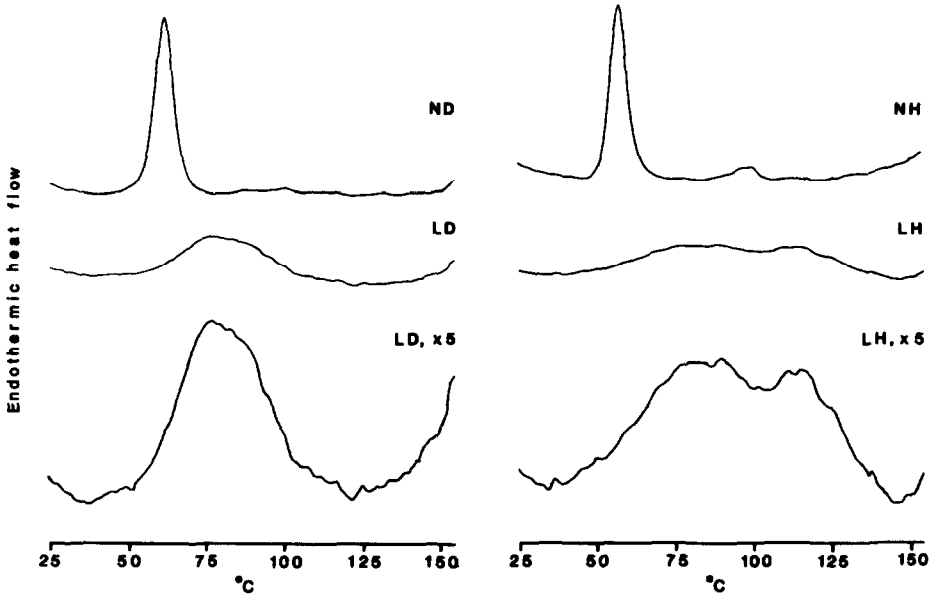

Fig. 5. DSC thermograms of native (N) starches and lintner residues (L); Dangomugi (D) waxy starch on left, Hector (H) non-waxy starch on right; the bottom thermograms are the same as the middle row (LD, LH) with ×5 scale expansion.

non-waxy starches (12–13.5 J/g and 8.4–10.4 J/g, respectively[4]), as would be expected following removal of non-ordered amorphous material by lintnerisation. The greater enthalpy for the non-waxy residues presumably reflects their higher double-helix content (Table III) and V-type conformation.

For both waxy and non-waxy residues, endotherm onset temperatures were similar to those of the native starches (42–46°C)[4], but the endotherm ranges were much greater (Fig. 5). For the waxy residues, the predominant event monitored by DSC was presumably disordering of double helices[20] formed from linear chains of modal CL16 (Fig. 1a). It is suggested that the greater enthalpy and higher final temperature may have been due to the presence of double helices involving longer chain segments than those formed in the precursor amylopectin, where geometric constraints imposed by clusters of branch points could serve to reduce the effective lengths of the helix-forming segments of the A and B_1 chains. An alternative possibility is that V-type conformations (discussed above) contributed to enthalpy values when disordered, or to the temperature of double-helix disordering by an anti-plasticisation action analogous to the effect of L · AM in raising the gelatinisation temperature of native starch granules[4].

The much narrower endotherm for the native starches illustrates the co-operative nature of the gelatinisation process, particularly as individual granules show even narrower temperature ranges for structure loss (birefringence) than the total granule population examined by DSC[5,20]. The covalent connectivity between

crystalline regions within amylopectin molecules is probably the origin of this effect, as severing these connections during lintnerisation decreases co-operativity dramatically (Fig. 5 and ref 45).

For non-waxy lintner residues, the DSC endotherm extended up to ca. 150°C (Fig. 5). In addition to disordering of short double helices (CL 16 chains), there would be disordering events for L · AM residues and for double helices formed from chains of modal CL46 (Figs. 1C and D). As there was no evidence from X-ray diffraction that the L · AM residues were crystalline (except in the lintner residues of ball-milled wheat starch which contained no residual helices from AP), disordering should have occurred in the range 94–100°C, and certainly not above 125°C[35,37]. Thus, the higher temperature range of the endotherm for non-waxy lintner residues is assigned to disordering of the double helices from residues of F · "AM".

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