SOME RECENT DEVELOPMENTS IN STARCH CHEMISTRY*

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Starch is a commercial product of great industrial importance. It has many uses in modern technology, but further expansion of these is very dependent on advances being made in our knowledge and understanding of the fundamental structural and physical chemistry of starch. One of the surprising aspects of starch chemistry is that, notwithstanding the immense amount of work which has been carried out during the past decades, many fundamental problems are not yet solved. Not least amongst these is the question of the nature and molecular structure of the granule. How do the two starch materials, amylose and amylopectin fit together in the granule? The problem is difficult. One essential approach is to find out more regarding the properties of the individual components. It is this aspect that I wish to deal with primarily in this paper, and shall briefly review: (1) some aspects of the fractionation of starch, into the component amylose and amylopectin, (2) recent investigations which characterize amylose more fully, (3) a recent enzymic technique for estimating the chain length of amylopectin, and (4) some problems which arise when dealing with granular starches.

1. The fractionation of starch

Starch can be fractionated into its component amylose and amylopectin in several ways, but the method which gives the most complete separation is that involving dispersion of the granules into aqueous solution, followed by complex-formation of the amylose with a polar, organic molecule such as butanol. Certain precautions are necessary to achieve maximum efficiency of this process: (1) the most important is that dispersion of the granules is as complete as possible to ensure good separation of the components, (2) the dispersion and fractionation should be carried out under oxygen-free conditions to avoid degradation and modification of the components, (3) the critical

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stage is the formation of the first amylose-complex, because the amylopectin — which is obtained from the resultant supernatant liquor — cannot be purified further; in contrast, the amylose-complex can be reprecipitated a further three or four times to achieve maximum purity; (4) the concentration of the dispersion should be low (not greater than 0.5% w/v); (5) the components should not be isolated in the solid state, but the properties of the amylose are best measured on the amylose-complex, whilst the amylopectin need not be isolated directly from the original supernatant liquor.

Table 1

Fractionation scheme for dimethylsulphoxide-pretreated potato starches

Potato starch granules				
+				
Dimethylsulphoxide				
↓				
Starch dispersion				
+				
Ethanol				
↓				
Precipitated non-granular starch				
H_2O at 20°C ↓	H_2O at 60°C ↓	H_2O at 70°C ↓	H_2O at 100°C ↓	NaOH at 0°C; HCl to p_H7 ↓
Dispersion + Butanol ↓	Dispersion + Butanol ↓	Dispersion + Butanol ↓	Dispersion + Butanol ↓	Dispersion + Butanol ↓
Amylose, A–20[a]	Amylose, A–60[a]	Amylose, A–70[a]	Amylose, A–100[a]	Amylose, A–OH[a]

[a] The corresponding amylopectin was obtained from the supernatant liquors after removal of the amylose-complex.

Obviously the whole success of the fractionation procedure depends on the effective dispersion of the granule. Our unpublished experiments have shown that this dispersion is a very complicated process. Experience shows that starches from certain botanical sources are more resistant to apparent dispersion than others. For example, root and tuber starches are more readily and successfully fractionated than those from cereals. In the past, we have treated such resistant granules with liquid ammonia to make dispersion easier [1]. We found this technique to be applicable to starches from a wide range of botanical sources [2]. More recently we have found that treatment with dimethyl sulphoxide (DMSO) is more convenient and effective. The dispersion of cereal starches into this solvent is particularly good.

Our attention was drawn to this solvent by the work of KILLION and FOSTER [3]. These authors reported that potato starch, fractionated after

dispersion into DMSO, yielded amylose of higher molecular weight and β-amylolysis limit (see below). Repetition of this work, however, failed to substantiate these claims [4]. We fractionated potato starch, which had been treated with DMSO from dispersions formed at temperatures from 20°C to 100°C as shown in Table 1. The properties of the different amyloses formed

Table 2

Properties of amyloses obtained from potato starch granules treated with dimethylsulphoxide

Fraction[a]	$[\beta]^{d}$	$[\beta+Z]^{d}$	$[\eta]$	% of amylose in amylopectin[b]
var. Redskin				
A—20	79	99	540	0.8
A—60	82	101	560	1.0
A—70	84	100	550	1.0
A—OH	82	99	540	0.8
A[c]	80	100	550	0.8
var. Pentland Crown				
A—20	85	99	620	0.8
A—60	85	99	690	1.0
A—70	85	102	640	0.3
A—100	86	101	630	0.5
A[c]	88	100	630	0.8

[a] Amylose obtained as in Table 1.
[b] From measurements of iodine affinity.
[c] Amylose from conventional dispersion at 100°C in water: no pretreatment.
[d] Percentage convention into maltose under the action of (i) β-amylase [β], and (ii) the conjoint action of β-amylase and Z-enzyme [$\beta + Z$].

are shown in Table 2, where they are comparable with those for an amylose from a conventional fractionation. It can be seen that all the amyloses have essentially the same β-amylolysis limits and size — as shown by the limiting viscosity numbers, $[\eta]$ — and there was no evidence that the samples from the pretreated starches were of larger molecular size, or contained a smaller number of structural anomalies. However, these experiments did demonstrate the efficiency with which DMSO will disrupt the granular structure, i.e. potato starch will then disperse into water at 20°C. We have since used a DMSO-pretreatment for many normally-resistant starches with great success, and would now recommend this as a standard procedure prior to the fractionation of any starch to ensure good dispersion into aqueous media.

2. The characterization of amylose

In our opinion, one of the most important characteristics of an amylose sample, which has been obtained from a dispersion as outlined above, is that the glucan is incompletely degraded into maltose by the enzyme, β-amylase. Typical results for samples of amylose from a wide variety of starches [2] are shown in Table 3. It can be seen that the β-amylolysis limit varies from about 70% to 85%, but is never the 100%, which is expected for a completely

Table 3

Typical β-amylolysis limits for amyloses obtained from starches of different botanical sources [2]

Amylose	$[\beta]^a$	$[\eta]$	Amylose	$[\beta]^a$	$[\eta]$
Barley	74	355	Potato	76	410
Oat	78	425	Apple	84	200
Wheat	72	330	Parsnip	72	590

[a] β-amylosis limit.

linear α-1:4-glucan. Elsewhere [5], we have assembled the evidence which shows that this β-limit is real, and represents some form of structural anomaly in the molecule.

Amylose in the starch granule is undoubtedly *heterogeneous* in nature, and consists of a spectrum of molecular types. The simplest method of demonstrating this is to subject starch granules to aqueous leaching at varying temperatures. Table 4 shows results which are typical of such a procedure. At low temperatures, the amylose-product is of low molecular weight, as shown by the viscosity, and is essentially *linear* for the β-amylolysis limit is 100%. In contrast, as the leaching temperature increases, the amylose increases in size and the β-amylolysis limit falls indicating that some structural anomaly is being introduced into the molecule. The behaviour is shown by starches from all botanical species. It has to be stressed that by both potentiometric iodine titration and enzymic characterization, all the subfractions are pure amylose and contain *no* amylopectin.

The heterogeneity in the properties of amylose can also be shown in other ways. For example, the amylose obtained from a conventional dispersion-fractionation can be subfractionated by dissolving it in DMSO, and reprecipitating the polysaccharide, in a stepwise manner, with ethanol or butanol. The subfractions obtained show a similar trend in β-amylolysis limit and

viscosity; the low molecular weight fractions have a high (100%) limit, whilst the larger fractions have a much lower limit.

Because such a subfraction process is causing separation of the polymer on the basis of differences in *both* molecular size and structure, the fractions obtained may not necessarily be homogeneous. This is demonstrated when

Table 4

Properties of amylose fractions obtained on successive aqueous leaching of some starch granules [4]

Starch	Procedure	Amylose extracted[a] (%)	[β]	[η]* (dl g^{-1})
Iris (rhizome)	70°C leach	19	98	190
	80°C leach	25	89	230
	90°C leach	25	76	260
	Dispersion of residue[b]	31	72	280
Potato	58—60°C leach	35	99	250
(var. Redskin)	63—65°C leach	15	82	320
	Dispersion of residue[b]	50	75	570
Wheat	70°C leach	26	98	145
	98°C leach[c]	81	69	260

[a] From measurements of iodine affinity.
[b] After addition of thymol and butanol as precipitants.
[c] Direct, and not successive, leach.
* Measured in 1 M potassium hydroxide.

the subfractions obtained with a low β-amylolysis limit are examined in the ultracentrifuge in dilute aqueous potassium chloride solution. The subfraction shows two distinct sedimenting species: a major slow-moving component and a smaller fast-moving component. Typical results [4] are shown in Table 5 where the heterogeneity of the sub-fractions with a low β-limit contrasts to the homogeneity of those with a high-value.

We believe, therefore, that this "amylose" obtained by fractionating starch is not structurally homogeneous, i.e. all the molecules are not linear α-1:4-glucans. The question remains as to the nature of this structural anomaly. We have discussed this in detail [5], and would suggest that the evidence is that some amylose molecules are branched; this branching is long-chain and there may be hundreds of glucose residues between the branch points.

Early hydrodynamic studies [6] first indicated that branching was occurring. The molecular weight (M_w) of fractions with a structural anomaly — that is, a low β-limit — did not lie on the same log M_w – *versus* – log [η]– graph as the linear fractions (β-limit $\approx$ 100%); for the same M_w-value, their viscosity was lower, indicative of branching. Enzymic experiments using the enzyme, pullulanase [7], have recently confirmed this view. This en-

zyme is specific for α-1:6-linkages and will, in fact, debranch amylopectin. If the structural anomaly in amylose is a result of branching then an α-1:6-branch point appears to be very likely. We treated amylose and the β-limit dextrin with pullulanase [8], and studied the effect of the enzyme on the susceptibility of the amylose to β-amylase either by successive action, or concurrent action. Table 6 shows that the successive action of these enzymes causes

Table 5

Typical apparent sedimentation coefficients for potato subfractions dissolved in 0.16 M potassium chloride at a concentration of 0.2 g/100 ml

Fraction	$[\beta]^{a)}$	$S_S{}^{b)}$	$S_F{}^{b)}$	Fraction	$[\beta]^{a)}$	$S_s{}^{b)}$	$S_F{}^{b)}$
PA1	85	9.5	17.4	PE1 a	70	11.2	
PA2	92	11.0	*	PE1 b	73	12.9	23
PA3	99	12.4	—	PE1 c	98	14	—
PA4	100	8.2	—	PE2 a	82	15	*
PA6	99	7.9	—	PE2 b	80	11	26

[a] β-amylolysis limit.
[b] S_S = sedimentation coefficient of slow component; S_F = sedimentation coefficient of fast component.
* Not measurable, minor peak present as a shoulder.

a reduction in $[\eta]$ and an increase in β-limit, $[\beta]$, but conversion into maltose is not complete. To ensure that debranching and not hydrolysis of 1 → 4 bonds was occurring, calculations were made of the theoretical β-limit, $[\beta]_{th}$, to be expected if the changes in $[\eta]$ were due to random degradation. It can be seen that $[\beta]_{th}$ is always less than $[\beta]$ showing that random degradation cannot cause the effect. Additionally, the concurrent action of the two enzymes on both the amylose and its β-limit results in essentially complete conversion into maltose. We thus conclude that α-1:6-branch points are present in amylose, and that these form the natural barrier to β-amylolytic action. Furthermore, the observed changes in $[\eta]$ are not extremely large, which suggests that the branches are long-chain rather than short-chain.

In summary, we regard the amylose component of the starch granule to consist of a mixture of molecules, the smaller ones being all linear, whilst, with increase in molecular size, there is an increasing possibility that the molecule has limited, long-chain branching. The completely linear molecules can be isolated by either aqueous leaching of the granule, or sub-fractionation of the total amylose.

The simplest method of characterizing the molecular size of amylose is by measurement of the limiting viscosity number, $[\eta]$, where $[\eta] = \lim_{C \to 0} (\eta_{sp}/C)$.

Table 6

Action of pullulanase and β-amylase on amylose samples

Amylose	Digest conditions [a]	Test			Control	
		$[\eta]$	$[\beta]$ [c]	$[\beta]_{\text{th}}$ [b]	$[\eta]$	$[\beta]$ [b]
Potato 1	1	210	99	98	230	96
Potato 2	1	440	97	88	670	86
Wheat 1	1	130	85	80	145	78
Wheat 1 β-limit dextrin	1	160	59	—	185	3
Wheat 2	1	110	88	80	135	76
Potato 1	2	—	100	—	—	96
Potato 2 β-limit dextrin	2	—	100	—	—	2
Wheat 1	2	—	97	—	—	78
Wheat 1 β-limit dextrin	2	—	97	—	—	2
Wheat 2	2	—	98	—	—	76

[a] 1 = successive action of pullulanase and β-amylase: 2 = concurrent action of these enzymes.
[b] β-amylolysis limits expressed as % conversion into maltose.
[c] Theoretical β-amylolysis limits calculated on the basis of random degradation.

There is a problem, however, in the choice of the most suitable solvent for this determination. We have studied the direct use of the butanol-complex in dilute potassium hydroxide [9], and found that the apparent $[\eta]$-value is very dependent of the strength of alkali used. At 1 M KOH and 10^{-2} M KOH, the measured $[\eta]$ is low, but it reaches a maximum at 0.15 M of alkali. We attribute this effect to ionization of the primary and secondary hydroxyls in the sugar ring. Ionization is a maximum in 0.15 M alkali, and hence the amylose molecule then expands to its maximum size due to repulsions between the ionized hydroxyls. We thus regard 0.15 M alkali as the most suitable solvent for a rapid characterization of molecular size of amylose. (Alkaline degradation is negligible in the time of measurement.)

The number-average molecular weight of *linear* amyloses can be conveniently and accurately estimated by enzymic assay. The basis of this method is as follows:

$$G-(G_{n-2})-G \xrightarrow[\beta\text{-amylase}]{\text{Excess}} n/2 \cdot G_2$$

$$G-(G_{n-1})-G \xrightarrow[\beta\text{-amylase}]{\text{Excess}} n/2 \cdot G_2 + 1 \cdot G$$

Any glucan with an odd-number of glucose residues will give rise to one molecule of glucose on β-amylolysis. This glucose can be readily estimated by glucose oxidase. Statistically, it is to be expexted that a glucan will contain

equal numbers of odd- and even-numbered molecules, and hence the number-average degree of polymerization DP_n can be readily calculated from the amount of glucose formed. We have described in detail the conditions for this analysis [10], and have shown that the accuracy of the technique is high. A typical test is shown in Table 7, where the agreement between the experimental and theoretical values for various synthetic mixtures of degraded amyloses is seen to be good. We have found that degrees of polymerization for linear amyloses of the order of 1000 can be determined to within 5%. Indeed, we regard this method as being the most successful of the direct ways of measuring the molecular weight of amylose.

Table 7

DP_n for mixtures of acid-degraded amylose

W_A [a]	W_B [a]	Exptal DP_n	Calculated DP_b [b]
1.000	0.000	386	—
0.946	0.054	110	109
0.895	0.105	63	65
0.781	0.219	32	34
0.486	0.514	14.5	15.2
0.000	1.000	8.0	—

[a] Weight fraction of component
[b] Calculated from $DP_n = 1/\Sigma(W_i/DP_i)$ where W_i and DP_i are the weight fraction and degree of polymerization for any particular species, i.

3. Characterization of amylopectin

Many problems regarding the detailed structure of amylopectin remain unsolved, but even the determination of the average length of unit chain is not straightforward. This determination is usually carried out by means of periodate oxidation, but this method has disadvantages. In particular, it is difficult to apply adequate corrections for over-oxidation, and for the presence of contaminating, amylose-type material. We have found such difficulties to be very pertinent in our recent studies of amylomaize starch and its sub-fractions.

A recent enzymic method of chain-length determination has proved to be an invaluable advance in technique. Again use is made of the enzyme, pullulanase. The branched glucan is degraded by the concurrent action of this enzyme — which removes the α-1 : 6-linkages — and a high concentration of β-amylase. Under these conditions, chains with an even number of glucose

residues will be converted into maltose, whilst chains with an odd number of residues will be converted into maltose and *one* glucose molecule (arising from the maltriose). This glucose can be specifically estimated by glucose oxidase, and hence the average length of unit-chain of the amylopectin can be calculated on the basis that the polysaccharide has equal numbers of even and odd lengths of chain. Typical results [11] obtained by this method are given in Table 8. It can be seen that agreement between the periodate oxidation method and this enzymic assay is good except for samples with a high iodine affinity. However, this is a case where the correction necessary for the periodate oxidation results is not known exactly, and we place more reliance on the enzymic assay.

Table 8

Comparison of average chain-lengths of amylopectin samples by enzymic and periodate assay

Sample[1]	Iodine affinity	Chain-length		Sample[2]	Iodine affinity	Chain-length	
		Enzymic	Periodate			Enzymic	Periodate
Barley	0.60	20	20	Amylomaize 1	2.30	34	38
Maize	0.10	24	27	Amylomaize 2	4.20	45	37
Oats	0.50	19	18	Amylomaize 3	0.12	27	—
Potato	0.02	22	26	Amylomaize 4	0.19	27	28
Waxy maize	0.04	17	23	Amylomaize 5	20.8	240	—
Wheat	0.50	21	20	Amylomaize 6	20.2	250	—

[1] Amylopectin prepared from laboratory-extracted starches by removal of the thymol-amylose complex from aqueous dispersions.
[2] Fractions of amylomaize starches.

We have found that the enzymic method gives more reproduceable results. Its inherent specificity is an added advantage, and we now use that method routinely.

4. Some problems in the study of granular starches

One important feature of whole starches, which has only recently been fully appreciated, is that all the granular properties depend on the stage of maturity of the original plant-material. For example, as the plant grows there are the following general changes in the nature of the starch: (a) the granules increase in size; their gelatinization temperature falls; and the percentage of amylose in the granule increases; and (b) the fractionated, component amylose and amylopectin increase in molecular size, and the β-amylolysis limit of the amylose decreases. Table 9 shows typical results [12] for these

changes in molecular properties found for the components of potato starch as the potato tuber grows (samples 1—13 represent increasing maturity).

The existence of this effect means that comparisons of procedure, technique, or measurements of physical or chemical characteristics cannot be made except on the same sample of starch. This phenomena also obviously accounts for many of the apparent discrepancies in the literature.

Table 9

Properties of the amylose and the amylopectin components isolated from dispersions of the starches

Iodine titrations showed that all the amylose samples were > 98% pure, and all the amylopectin samples were > 99.7% pure

	Amyloses			Amylopectins			
Starch sample	β-Limit [a]	[η] (C in g/ml)	Degree of polymerization	β-Limit [a]	Chain-length	Internal chain-length [b]	Molecular weight (×10⁻⁶)
1	92	305 [c]	2200	56	26	9	9
2	90	145	1100	56	26	9	n.d.
3	90	185	1400	55	26	9	n.d.
4	88	210	1600	54	24	9	23
5	88	276	2100	54	23	8	n.d.
6	87	345	2800	53	23	8	n.d.
7	85	415	3100	52	23	9	35
8	84	435	3200	52	24	9	n.d.
9	84	450	3300	51	23	8	n.d.
10	83	490	3600	51	22	8	37
11	83	505	3700	52	22	8	n.d.
12	81	540	4000	51	22	8	60
13	81	530	3900	52	22	8	130

[a] Percentage conversion into maltose on treatment with β-amylase.
[b] Calculated from {chain-length — [(chain-length × β-limit) + 2.5]}, to nearest whole number.
[c] Average from two independent fractionations when [η] = 300 and 310, respectively.
n. d. = not determined.

Our most recent investigations involving granular starches have been concerned with those of high amylose-content. Again, more problems remain than have yet been solved. It appears to us that the so-called high amylose-content maize starches do not contain their reputed large amounts of normal amylose, but contain a large amount of degraded material [13].

Much more investigation is required in this particular facet of starch chemistry — as in many others!

Summary

An account is given of some recent developments in starch chemistry. Problems in the dispersion and fractionation of granular starches are first discussed. Methods of characterizing the amylose component in terms of its β-amylolysis limit, structural heterogeneity, and molecular size are then outlined with an emphasis on enzymic investigations — including a new method to give number-average molecular weights. The importance of an enzymic technique for characterizing amylopectin is stressed. The properties of starch granules are shown to depend entirely on the maturity of the plant source. Finally, some problems in the chemistry of starches of high amylose-content are outlined.

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