Note

Mild oxidation of starches with aqueous bromine
Part II. Solution viscosity of the oxidised starches*

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In the course of an investigation of the mild action of aqueous bromine on amylose and amylopectin, the oxidation kinetics and the functional group content of the oxystarches formed have been reported[2] The extent of molecular degradation due to the oxidation was studied by the method of solution viscosity, and the results are now described

Following oxidation at pH 8, both amylose and amylopectin exhibited a ten-fold decrease in intrinsic viscosity $[\eta]$, and a considerable but highly variable rise in slope constant k' (Tables I and II) In the case of amylose in M potassium hydroxide, $[\eta]$ is approximately a linear function of both weight-average and number-average molecular weights, as shown in the Mark–Houwink equations[3,4], which yielded values of *ca* 500,000 and 165,000 for $\overline{M}_w$ and $\overline{M}_n$, respectively, of untreated amylose If these equations are assumed to be applicable to the low values of $[\eta]$ obtained for the oxyamylose (Table I), the oxidation causes a ten-fold degradation The intrinsic viscosity of amylopectin is less sensitive to changes in molecular weight than amylose[5], so that the oxidation also led to a drastic decrease in the molecular weight of amylopectin

Carbohydrates are sensitive to atmospheric oxidation and degradation in alkaline media It was therefore necessary to consider the possibility that the considerable decreases in molecular weight were not directly due to bromine oxidation The carbonyl contents[2] and viscosity data for products of control runs in the absence of oxidant were similar to those for untreated materials, showing that atmospheric oxidation and subsequent degradation were insignificant during our preparation of oxystarches.

Oxystarches prepared at pH 7 had, if anything, a slightly lower intrinsic viscosity than pH 8 samples, indicating that the oxystarches do not undergo alkaline degradation at pH 8 Furthermore, the flow-times for solutions of these oxystarches in M potassium hydroxide were practically unchanged during many hours (see Table III), so that their stability is maintained even in this highly alkaline medium

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TABLE I

VISCOSITY DATA FOR OXYAMYLOSES[a]

Batch No	*Oxidation conditions*		*Borohydride treatment (h)*	$[\eta]$ *(dl/g)*	k'
	pH	*Oxidant consumption (mmoles/162 g)*			
1	Untreated material			1 29	0 34
				1 35	0 32
	8	nil[b]		1 47	0 32
		47 6		0 21	1 20
		47 6		0 21	0 93
		48 8		0 20	1 64
		71 4[c]		0 14	2 22
	7	nil[b]		1 42	0 31
		38 9		0 16	1 13
		46 1		0 16	0 28
2	Untreated material			1 43	0 29
				1 36	0 39
			24	1 20	0 29
			24	1 20	0 58
	8	62 5		0 16	2 03
				0 14	3 53
			24	0 45	0 42
			48	0 45	0 42

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[a] Oxidations performed on 5 4 g of amylose with 2 5mM bromine (1 litre), unless otherwise specified [b] Control run in absence of oxidant [c] Oxidant 5 0mM bromine

TABLE II

VISCOSITY DATA FOR OXYAMYLOPECTINS[a]

Oxidation conditions		*Borohydride treatment (h)*	$[\eta]$ *(dl/g)*	k'
pH	*Oxidant consumption (mmoles/162 g)*			
Untreated material			1 37	0 99
			1 22	1 04
		24	1 13	0 85
		24	1 19	1 00
8	nil[b]		1 18	1 23
	20		0 24	2 28
	21 4[c]		0 23	2 97
	21 7[c]		0 21	3 21
	21 7[c]	24	0 65	1 32
	39 1		0 17	1 80
	44 2		0 16	2 35
7	nil[b]		1 03	1 37
	40		0 14	2 06
	43 5		0 14	2 27
	43 5	24	0 34	2 37

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[a] Oxidations performed on 5 4 g of amylopectin with 2 5mM bromine (1 litre), unless otherwise specified [b] Control runs in absence of oxidant [c] 11 g of starch oxidised

TABLE III

EFFECT OF STORAGE ON VISCOSITY

Sample	*Conc (g/dl)*	*Storage time*[a] *(h)*	*Flow times (sec)*	
			Initial	*Final*
Untreated amylopectin	0 500	24	588 7	576 1
Untreated amylose	0 948	24	686 5	676 0
	0 506	4	463 9	441 0
pH 7-Oxyamylose[b]	0 514	4	271 0	267 0
	0 257	3 5	259 5	257 9
	0 128	24	251 5	250 0
pH 7-Oxyamylose[b]	1 08	24	301 2	290 6
	1 01	64	298 6	293 4
	0 27	24	257 4	257 4

[a]Storage in closed vessel under air at room temperature [b]Samples listed in Table I

Reduction of the oxystarches with sodium borohydride results in a drop in the carbonyl group content as indicated[2] by reaction with sodium cyanide and by infrared spectroscopy After reduction with borohydride, the oxidised starches showed a dramatic rise in intrinsic viscosity, and the slope constant was, in general, lower and approaching the value for unoxidised starch The increased [η] after treatment with borohydride could be ascribed to the presence of borate in the solution used for viscosity determination, since borate is known to complex with polyhydroxylic compounds and to form cross-links This possibility was investigated, and the results are summarized in Table IV In the presence of borate, oxyamylose had only a slightly enhanced intrinsic viscosity, whereas borohydride-reduced oxyamylose retained its high intrinsic viscosity after removal of borate It was concluded that the high values for reduced oxystarch were not an artifact This implies that the oxystarches undergo *rapid* degradation during dissolution in M alkali, since thereafter the intrinsic viscosity did not decrease significantly for many hours (Table III) This rapid degradation was absent after reduction of carbonyl groups Therefore, bromine-oxidised starches must be suitably reduced before measurements of molecular size are performed in aqueous alkali

The exponent α in the Mark–Houwink equation for amylose in 0 15M potassium hydroxide is 0 77, indicating macromolecular extension by long-range solvent effects[6] When the alkalinity of the solvent is raised to M, the value of α approaches unity[3–5] and [η] drops[6] As a result of their interaction with alkali, starch molecules exist as extended polyanions in this solvent[6] In oxidised starch, the carboxylate groups will also contribute to the molecular charge in alkali, thereby affecting the extent of molecular expansion and the intrinsic viscosity

The presence of carbonyl (aldehyde and ketone) functions in an oxidised starch, however, has a more-profound influence on viscosity parameters Periodate-oxidised starch rapidly degrades in alkali by the beta-alkoxycarbonyl mechanism, but reduction

TABLE IV

INFLUENCE OF BORATE ON VISCOSITY PARAMETERS

	$[\eta]$ (*dl/g*)	k′
pH 8-Oxyamylose		
1 dissolved in alkali	0 16, 0 14	2 03, 3 53
2 stirred for 24 h with aged borohydride[a] before addition of, and dissolution in, alkali	0 21	1 00
3 dissolved in alkali + fresh borohydride	0 19	1 08
4 dissolved in alkali + borate	0 18	1 30
Borohydride-reduced pH 8-oxyamylose[b]		
1 dissolved in alkali	0 38	0 55
2 dissolved in alkali + borate	0 37	0 56
3 acetic acid added and repeated distillation of methanol before dissolution in alkali	0 38	1 49

[a] Aqueous solution of sodium borohydride stored for 24 h [b] After reduction for 24 h, centrifugation, and washing with water and with methanol

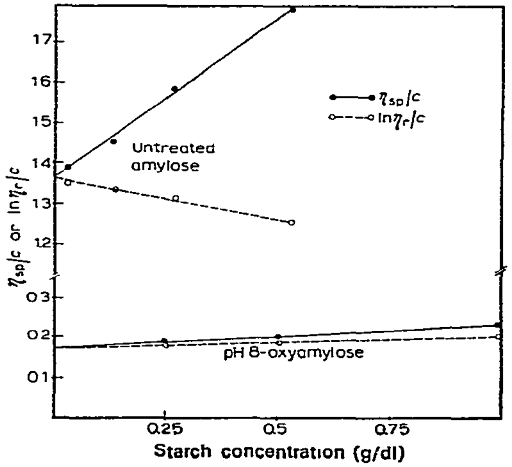
with borohydride prevents this reaction and permits the determination of values for intrinsic viscosity[7] Hypochlorite-oxidised starches were reported to be unaffected by borohydride reduction in so far as the intrinsic viscosity in M alkali is concerned[7] Our data indicate that bromine-oxidised starch has alkali-labile carbonyl groups, which result in rapid chain-scission in alkali If the beta-alkoxycarbonyl mechanism is involved, the active carbonyl groups will be on C-2, 3, or 6 of the D-glucose residues A "peeling "reaction from terminal, reducing end-groups would not cause a rapid degradation and is not indicated by the data in Table III

The extensive depolymerisation observed during oxidation may be the result of beta-alkoxyl elimination reactions engendered by ketone groups at C-2 or C-3, or by an aldehyde or carboxyl function at C-6 It may be suggested, however, that chain scission occurs in an analogous fashion to the chlorinolysis of the C-1 bond to glycosidic oxygen[8]

In a study of periodate-oxidised amylose and amylopectin, Erlander has correlated raised values for *k′* with formation of aggregates[9], which are held together by hydrogen bonds[10] Our results for bromine-oxidised starch components also indicate raised values for *k′*, which tend to decrease after reduction However, anomalous values can often be obtained as a result of the experimental error at low $[\eta]$

EXPERIMENTAL

The preparation of the oxidised starches has been described previously[2] All solutions were filtered through a No 4 glass sinter Clear solutions of starch (1 or 0 5%) were prepared in filtered, aqueous potassium hydroxide (1 01M) under nitrogen with magnetic stirring during 15 min The filtered solution was kept at 30° in a closed flask, and dilutions were performed with filtered alkali A No 50 Cannon–Fenske viscometer (water flow-time *ca* 240 sec) was used at a bath temperature of 30 ±0 01° Starch concentrations were calculated from the dry weight of the sample taken and are given in units of g/dl Kinetic corrections were not applied



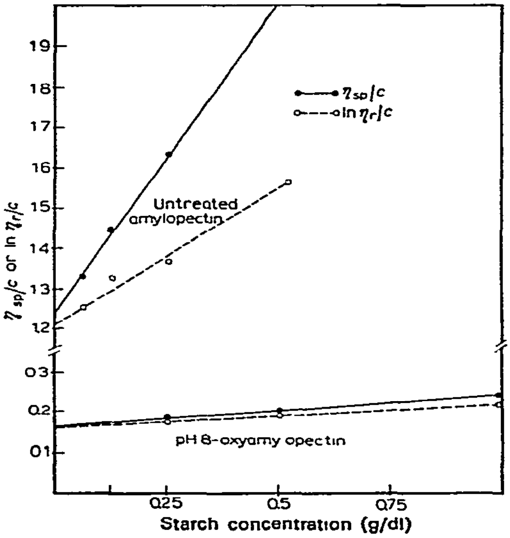


Fig 1. Viscosity of amylose and pH 8-oxyamylose in M potassium hydroxide

Fig 2 Viscosity of amylopectin and pH 8-oxyamylopectin in M potassium hydroxide

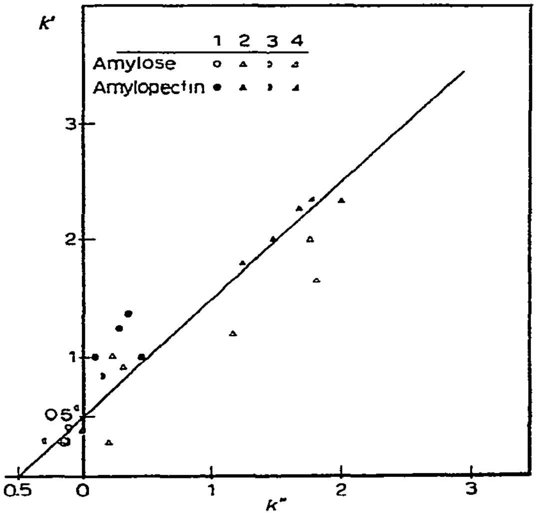


Fig 3 Slope constants k' and k'' from viscosity data $\eta_{sp}/c = [\eta] + k'[\eta]^2 c$ $\ln \eta_r/c = [\eta] + k''[\eta]^2 c$, Straight line for equation $k' = k'' + 0\ 5$ 1, starch 2, oxystarch 3, borohydride-treated starch 4 borohydride-treated oxystarch

The borohydride treatment was carried out as follows freshly prepared, aqueous sodium borohydride (6 2mM, 20 ml) was added to *ca* 225 mg of starch, giving an 8-fold molar excess of hydrogen equivalents per starch carbonyl group The suspension was stirred magnetically for 24 h at room temperature, after which time aqueous potassium hydroxide (10 6M, 4 ml) followed by water (20 ml) was added, final concentrations were 0 95M potassium hydroxide and 2 8mM sodium borohydride The solution was filtered before viscometry Dilutions were performed with alkaline borohydride solution prepared identically

Treatment of data — In order to obtain values for intrinsic viscosity and slope constant k', a double plot was first tried by using the Huggins[11] equation *1* and a modified Kraemer[12] equation *2*

$$\eta_{sp}/c = [\eta] + k'[\eta]^2 c \qquad (1)$$

$$\ln(\eta/\eta_0)/c = [\eta] + k''[\eta]^2 c \qquad (2)$$

Extrapolations of this double plot to zero concentration gave a common intercept, *viz* $[\eta]$ The slope of the logarithmic plot was negative for unoxidised amylose, but positive for amylopectin and oxystarches (Figs 1 and 2) When the slope constants k' and k'' were calculated according to equations *1* and *2*, the values were not strictly in accordance with relationship *3* (see Fig 3)

$$k' = 0\ 5 + k'' \qquad (3)$$

This result is explained by Sagai[13], who showed, by mathematical analysis of equations *1* and *2*, that k'' is an unreliable measure of the Huggins slope-constant, its value is a variable function of k', so that equation *3* is inapplicable Consequently we have adopted Sagai's procedures[13] for approximating correct values for both $[\eta]$ and k' For good-solvent systems, this involves using the arithmetic means of values calculated from linear extrapolations based on the Martin[14] (equation *4*) and Huggins[11] (equation *1*) plots

$$\ln(\eta_{sp}/c) = \ln[\eta] + k'[\eta]c \qquad (4)$$

The results thus calculated are given in Tables I and II for amylose and amylopectin, respectively

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