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The role of the structure on the molecular size and conformation of (1-4) and (1-6) linked polysaccharides

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The elution behaviour on gel chromatography of native and partly methylated galactomannans in neutral salt and alkali, and of highly methylated galactomannans in chloroform and neutral salt was very similar, indicating that O-5 to 3-OH hydrogen bonding is not required for maintaining an extended structure in aqueous solution. The large decrease in viscosity and increase in elution volume that accompanied periodate oxidation and borohydride reduction under conditions of nearly quantitative uptake of periodate, as well as undetectable depolymerisation, were consistent with a major conformational change resulting from conversion of the repetitively internally-bridged structure of the original glycan, in which all but one of the atoms of the chain are unable to rotate, to the dioxo-trimethylene structure, in which free rotation of all atoms is possible. The decrease in molecular size of the periodate-oxidised borohydride-reduced derivatives allowed their elution behaviour to be compared with protein standards.

INTRODUCTION

Calculations of the favoured conformation of β(1-4) pyranosyl linked polysaccharides, where the ring linkages involve two equatorial bonds, as found in D-glucosyl and D-mannosyl polymers (glucomannan, galactomannan, xyloglucan and galactoglucomannan) indicate a very extended helical structure, (Sandararajan & Rao, 1970; Rees & Scott, 1971; Atkins *et al.*, 1973; Burton & Brant, 1983). In the solid state extended structures have been described from X-ray and electron diffraction studies for a number of β(1-4) polymers, such as cellulose (Marchessault & Sundararajan, 1983), (1-4) β-mannan (Chanzy *et al.*, 1987; Atkins *et al.*, 1988) and (1-4) β-glucomannan (Yui *et al.*, 1992). This conformation is also found in substituted β(1-4) chains such as galactomannans (Song *et al.*, 1989). There is considerable evidence that in aqueous solution molecules of this type (e.g. galactomannan, glucomannan, galactoglucomannan and xyloglucan) retain aspects of this extended structure (Brant, 1980; Rees *et al.*, 1982). A recent comparison of chiroptical properties and theoretical calculations of conformation of β-mannan indicated that conformations of the solid-state are prevalent in solution (Duda & Stevens, 1992). These polymers characteristically show non-ideal behaviour on examination by physicochemical methods, consistent with a non-spherical shape. Light-scattering measurements indicate anisotropic molecules. On ultra-centrifugation, there is a strong dependence of sedimentation rate on concentration (Kubal & Gralén, 1948; McCleary & Matheson, 1975). The viscosities are high, with non-ideal flow behaviour, and on gel chromatography the molecules are excluded from all but matrices with the largest inclusion capacities (McCleary & Matheson, 1975; Mallett *et al.*, 1987), all properties shown by polymers with an elongated shape. High molecular size in solution can also be due to associative effects, which can be between like molecules (gellan (Chandrasekaran *et al.*, 1988), β(1-3) xylan (Atkins *et al.*, 1969)) leading to a coiled multihelical structure, or between unlike molecules (locust bean galactomannan and agarose (Morris, 1986)). The non-ideal behaviour, which is a consequence of an extended conformation, can be contrasted to that of most soluble proteins that adopt a globular shape and have a population which is homogeneous in molecular weight. With these compounds molecular weight can be

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accurately determined by physical methods combined with a knowledge of the amino acid composition.

One way of viewing polysaccharides is to consider the sugar ring as the monomeric unit. Then the reasons that have been proposed for the extended shape of this group of polysaccharides include the resistance to rotation about the glycosidic bonds because this brings two neighbouring pyranose units into a structurally more hindered relationship, and hydrogen bonding between a 3-OH and the 5-O (ring oxygen) in a neighbouring residue. The properties resulting from this extended conformation are significant in biological function, e.g. plant cell wall structure, and in industrial use, e.g. food gums (Brant, 1980).

Other authors (Smidsrød & Painter, 1973; Casu *et al.*, 1982, 1985) have viewed the polysaccharide chain in a different way. A comparison of the viscosity of alginic acid, cellulose and amylose before and after periodate oxidation showed a very large decrease on periodate oxidation. It was proposed that the decrease was due to the increase in rotational flexibility of individual atoms in the chain that accompanied the opening of the pyranose ring. This modification of conformation should lead to alteration in molecular size which could be monitored by gel chromatography, when a more compact folded structure would be included in a smaller pore size, and by viscosity, since a more globular molecule would interact less with neighbouring molecules than a long extended structure. The oxidised product would then be more closely related in structural aspects to another class of biological polymers, the globular proteins.

In the present study the elution behaviour on exclusion chromatography of galactomannan and dextran in neutral and alkaline solution, before and after partial methylation and, before and after periodate oxidation followed by borohydride reduction, has been compared. The elution rate on exclusion chromatography of a range of (1-4) and (1-6) linked polysaccharides after periodate oxidation and borohydride reduction has been related to those of standard proteins of well-characterised molecular weight.

MATERIALS AND METHODS

Source and preparation of polysaccharides

Dextran T-2000 (Pharmacia), amylose (Sigma, potato type III, A0512), locust bean (*Ceratonia siliqua*) gum (Sigma, G0753), rabbit liver glycogen (Sigma, G4011) and tamarind seed xyloglucan (Megazyme, North Rocks, Australia) were commercial products. The composition of the xyloglucan was arabinose/galactose/xylose/glucose 3:16:36:45. Konjac glucomannan was prepared by extraction into 0·1 M NaCl of commercial konjac flour (*Amorphophallus konjac*) and the product isolated by centrifugation after precipitation from the supernatant with ethanol. The locust bean galactomannan fraction was prepared by wetting powdered locust bean gum with ether, adding water and stirring overnight. The mixture was then heated in a water bath at 40–50°C with stirring for 3 h, centrifuged and three volumes of ethanol added to the supernatant. The precipitate was washed with ethanol, acetone and ether and dried under vacuum. Fenugreek (*Trigonella foenum-graecum*) galactomannan was prepared from seed that had been soaked in 0·2 M NH_4OH for 1 h. The seeds were washed free of alkali and macerated in 0·1 M NaCl. After centrifugation (20 000 × *g* for 20 min) the supernatant was filtered through Miracloth and galactomannan precipitated with Fehling's solution and collected by centrifugation (4 000 × *g* for 10 min). The complex was suspended in water and 1 M HCl added dropwise until it returned into solution. The solution was dialysed against running tap water for 48 h and then distilled water for 10 h. Polysaccharide was precipitated in ethanol and washed with ethanol, acetone and ether and dried under vacuum to a powder. The β-limit dextrins of waxy maize and glycogen were prepared by incubation of these polysaccharides in acetate buffer (pH 5·0) with β-amylase (500U, sweet potato, Boehringer 102 822) in a dialysis bag against the same buffer overnight. More β-amylase (400U) was added for a further 24 h. The solution was dialysed against water for 30 h (with one change of water) the solution boiled, centrifuged and freeze-dried.

Protein chromatography standards

Apoferritin (A3660) thyroglobulin (T9145) and sweet potato β-amylase (A8781) were obtained from Sigma.

Preparation of lead dithionate

On removal of barium as the sulphate from barium dithionate by centrifugation after addition of sulphuric acid, lead carbonate was added and excess removed by centrifugation. Lead dithionate was then crystallised from solution by the addition of ethanol (O'Dea & Gibbons, 1953). Barium dithionate was prepared by reaction between manganese dioxide and sulphur dioxide below 10°C, filtration and reaction of the filtrate with barium hydroxide (Pfanstiel, 1946).

Preparation of chromotropic acid reagent

Chromotropic acid (1·0 g, Sigma-D5144) was dissolved in hot water (100 ml), filtered, 0·1 g stannous chloride added and the filtrate diluted to 500 ml with sulphuric acid (66% v/v) (O'Dea & Gibbons, 1953).

Methylation of polysaccharides

Dimethyl sulphate (70 ml) was added over a period of several hours to polysaccharide (5·0 g) in 30% aqueous

sodium hydoxide (oxygen-free) (300 ml) with the temperature kept below 25°C. Nitrogen (oxygen-free) was bubbled through the solution. After storing for a further 18 h 30% sodium hydroxide solution (200 ml) was added, followed by dimethyl sulphate (70 ml) over a period of 8 h and then the mixture stored overnight. After one more methylation under similar conditions the solution was slowly neutralised in an ice-bath with ice-cold 80% acetic acid and dialysed against flowing tap water for 24 h and then against distilled water with 2-hourly changes of water. The volume was reduced to 200 ml by concentrating the solution in a dialysis bag opposite a fan and the solution dialysed for a further 24 h and freeze-dried. A comparison sample of unmethylated polysaccharide was treated similarly but without the addition of dimethyl sulphate.

At this stage methylated dextran T-2000 was soluble in chloroform and a portion was extracted into chloroform to provide the chloroform-soluble fraction for chromatography. To obtain a chloroform-soluble sample of methylated fenugreek galactomannan this procedure was repeated twice (nine methylations) and the product extracted into chloroform.

Gel chromatography of unmethylated and partially methylated polysaccharides

Polysaccharide solution (2·0 ml, 0·1–0·2%) in 0·1 M Na_2SO_4 was applied to a column of Fractogel TSK HW-55(S) for amylose and konjac glucomannan, or Fractogel TSK HW-65(F) for dextran T-2000, tamarind seed xyloglucan and locust bean and fenugreek galactomannans and eluted with either 0·1 M Na_2SO_4 or 0·25 M NaOH. The column size was 72 × 1·60 cm and the flow rate 10·0 ml/h. About 50 fractions were collected and hexose content determined by the phenol–sulphuric acid procedure (Hodge & Hofreiter, 1962). Eluate (1·0 ml) was mixed with 5% phenol in 0·2 M H_2SO_4 (1·0 ml) and concentrated sulphuric acid (5·0 ml) added while mixing on a vortex mixer. A calibration curve with glucose (0–70 μg/ml) was also prepared and after 40 min absorbance was recorded at 490 nm.

Partially methylated polysaccharides were chromatographed and estimated in the same way.

Gel chromatography of methylated polysaccharide fractions soluble in chloroform

Methylated polysaccharide solution containing 3·5 mg/ml (2·0 ml) was applied to a column of Fractogel TSK HW 65(F) which had been previously equilibrated in chloroform and eluted with chloroform (column size 72 × 1·6 cm and flow rate 24 ml/h). Each fraction was weighed just after collection to avoid loss of chloroform by evaporation. The chloroform was evaporated in a steam bath and the residue dissolved in 0·25 ml NaOH to a concentration of 0–80 μg/ml and the glycan content measured by the phenol–sulphuric acid method. Glucose was routinely used as a standard but then the values were adjusted by the ratio of the absorption of tetramethyl glucose relative to glucose when estimated with phenol–sulphuric acid reagent. (Tetramethyl glucose gave 80% of the absorption reading of the same weight of glucose.)

Periodate oxidation followed by measurement of viscosity changes of polysaccharides

Polysaccharide solution (20·0 ml) in 0·05 M acetate buffer was equilibrated to 20·0°C in a Ubbelohde viscometer. Sodium periodate in the same buffer and at the same temperature (70 mM–5 ml) was added, the solution mixed rapidly and the flow rate measured at various times. Zero time flow rate was measured after adding 0·12 M acetate buffer instead of periodate in buffer. The viscometer capillary diameter was 0·5 mm and the time of flow with solvent 98·6 s.

Preparation of periodate-oxidised, borohydrice-reduced polysaccharides

Polysaccharide (25 mg) was wetted with ether and water added slowly with occasional mixing until the polysaccharide was dispersed into solution and the volume was 10·0 ml. Sodium periodate (150 mg) was added and the mixture gently stirred in the dark at 4°C for 18 h. 2,3-Dihydroxybutane (100 μl) was then added and the solution stirred for another 2 h. Lead acetate (8·56 M–0·80 ml) was added and the solution centrifuged. Powdered sodium hydrogen carbonate was carefully added to bring the pH to 6·3 and the solution centrifuged again. Potassium borohydride (10 mg) was added to the supernatant and the solution stored at room temperature for 24 h. This solution was used directly for chromatography.

Gel chromatography and detection of periodate-oxidised, borohydride-reduced polysaccharides

Oxidised-reduced polysaccharide solution (2·0 ml, 0·1–0·2%) was chromatographed on Fractogel TSK HW 55(S) (for dextran T2000, amylose, tamarind xyloglucan and konjac glucomannan) or Fractogel TSK HW 65(F) (for locust bean and fenugreek galactomannans or glycogen and waxy maize β-limit dextrins). The column size was 72·0 × 1·6 cm and the flow rate 10·0 ml/h. The oxidised-reduced polysaccharide was eluted with 0·1 M Na_2SO_4 solution and the content of the column fractions estimated as follows. An aliquot (0·10 ml) containing approximately 60 μg/ml of oxidised-reduced polysaccharide was added to water (0·9 ml) followed by freshly prepared 2,4-dinitrophenylhydrazine reagent (1·0 ml) (0·1% 2,4 dinitrophenylhydrazine (BDH) in 2 M HCl). After mixing and lightly stoppering, the tubes were heated in a boiling water bath for 45 min, cooled and

2 M NaOH in 70% ethanol (3·0 ml) added. After 20 min the absorbance at 550 nm was read. Standard curves were prepared with glycolaldehyde (0–2 μg/ml).

Determination of uptake of periodate and formaldehyde production by amylose

Amylose, dissolved in water (25 ml) was mixed with 0·14 M sodium periodate (25 ml) and stirred at 4°C in the dark. Periodate consumption was estimated after dilution with water of an aliquot to 15 mM with respect to sodium periodate which was then diluted 250-fold with water and absorption measured at 223 nm. For estimation of formaldehyde 20% w/v lead dithionate (1·0 ml) was added to an aliquot (1·0 ml) and the mixture centrifuged. Chromotropic acid reagent (9·0 ml) was added to an aliquot (1·0 ml) of the supernatant and after 40 min the mixture centrifuged. The supernatant was heated in a boiling water-bath for 40 min and cooled under running tap water. The absorption at 570 nm was read and compared with a calibration curve for formaldehyde (0–9 μg/ml).

RESULTS AND DISCUSSION

Two galactomannans, fenugreek (with a high galactose to mannose ratio (48:52)), the fraction of locust bean galactomannan soluble in warm water (with a low galactose to mannose ratio (24:76)) as well as dextran T-2000, composed of mostly (1-6) linkages, were partly methylated by three treatments with dimethyl sulphate in sodium hydroxide under nitrogen. Samples of the original polysaccharides were kept in alkali under the same conditions. The Haworth methylation procedure was chosen since it allowed the preparation of a comparison sample of unmethylated material. The unmethylated samples were chromatographed on a gel exclusion matrix (Fractogel TSK-HW 65-(F)) in neutral salt solution and the elution profiles compared to those in dilute sodium hydroxide. Carbohydrate contents of the eluted fractions were estimated with phenol–sulphuric acid reagent. Intramolecular hydrogen bonding between 3-OH and the neighbouring ring oxygen (O-5) would be less favoured in alkali, allowing the possibility of more flexibility, leading to more folding, with a consequent increase in the elution volume and thus a decrease in $-\log K_{WAV}$ as the conformation became less extended. $-\log K_{WAV} = -\log (V_{EW} - V_0)/(V_t - V_0)$ = molecular size/a constant, where V_t is the total volume of the column, V_{EW} the weighted-average elution volume, and V_0 the void volume. The constant is specific for each matrix. Provided a significant amount of material at the void or total volume is absent, $-\log K_{WAV}$ is approximately linearly related to molecular size. Some polysaccharides show associative effects, and again, if intermolecular hydrogen bonding were significant, it would be less likely to be present in alkali. The results are shown in Table 1 and Fig. 1 and indicate very similar elution behaviour for each polysaccharide in both solvents, consistent with hydrogen bonding being an unlikely requirement for maintaining the extended conformation. The effect of alkali on the elution of dextran T-2000 was similar with no significant change in elution volume. The partly methylated samples were then chromatographed on Fractogel TSK-HW 65(F) with sodium sulphate as the elution solvent and the results are shown in Table 1. Wherever methylation of a 3-OH occurred no hydrogen bond to a neighbouring O-5 could form, resulting in a capacity for increased flexibility leading to an expected decrease in apparent molecular size and hence an increase in the elution volume. The elution profiles for the methylated and unmethylated

Table 1. Comparison of the elution on Fractogel TSK-HW 65(F) of unmethylated and partly methylated polysaccharides in 0·1 M Na_2SO_4 and 0·25 M NaOH as solvents

Polysaccharide	Unmethylated (U) or methylated (M)	Solvent	$-\log K_{WAV}$
Fenugreek	U	Na_2SO_4	0·60
galactomannan	U	NaOH	0·61
	M	Na_2SO_4	0·61
Locust bean	U	Na_2SO_4	0·53
galactomannan	U	NaOH	0·53
	M	Na_2SO_4	0·51
Dextran T-2000	U	Na_2SO_4	0·38
	U	NaOH	0·38
	M	Na_2SO_4	0·44

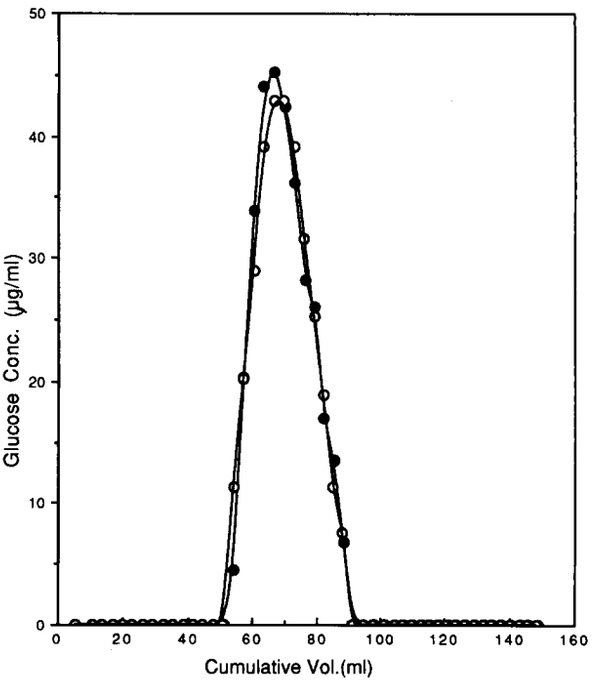

Fig. 1. Gel chromatography of unmethylated locust bean galactomannan in neutral salt and alkali. (●) 0·1 M Na_2SO_4; and (○) 0·25 M NaOH.

samples of the two galactomannans showed little difference. The dextran T-2000 sample showed a small increase. It appeared to undergo more methylation since it became completely soluble in chloroform. Full methylation would lead to an increase of approximately one quarter in molecular weight and this would be accompanied by an increased capacity for steric interaction between O Me groups on one pyranose ring and the next due to the conversion of hydroxyl to the larger methoxyl group.

The absence of any effect associated with a hydrogen bond between 3-OH and O-5 of the next pyranose ring is in accord with the prediction of the lack of a role for hydrogen bonding between hydroxyls (R-OH . . . O(H)R) acting as a stabilising influence on biopolymers in aqueous solution (Cox *et al.*, 1991). The equilibrium between the bonded and unbonded states in aqueous solution has been calculated to show negligible entropy and enthalpy changes. This is in contrast to other neutral bonds in biological molecules such as amide-hydroxyl bonds of proteins which are favoured entropically.

Fenugreek galactomannan was subjected to six further Haworth methylations to increase the solubility in chloroform. Then, after extraction with this solvent the major part was soluble. A control examining the effect of alkali on polysaccharide alone, which was monitored by viscosity change showed that the limiting viscosity number (in 0·1 M Na_2SO_4) decreased from 470 to 122 ml/g, indicating some degree of depolymerisation. The viscosity number of the chloroform-soluble methylated polymer (130 ml/g) was similar to that of the unmethylated control. This fraction soluble in chloroform was then chromatographed on Fractogel TSK 65(F) in chloroform and the elution behaviour compared with the same fraction in aqueous sodium hydroxide. The elution volumes were very similar (Table 2) indicating that even in chloroform no major change in conformation compared to that in water occurred. The results indicate that the molecular size of galacomannan is an inherent property of the molecular structure and not dependent on the aqueous environment. With dextran T-2000 the $-\log K_{WAV}$ value was lower in chloroform indicating a decrease in the molecular size in this solvent compared to sodium hydroxide. The increased flexibility due to the presence of two atoms in the chain being capable of rotation may have resulted in a significant response to the change of solvent.

Table 2. Comparison of elution on TSK-HW 65(F) of chloroform-soluble methylated polysaccharides in alkali (0·25 M NaOH) or chloroform as solvents

Polysaccharide	Solvent	$-\log K_{WAV}$
Fenugreek galactomannan	$CHCl_3$	0·19
	NaOH	0·22
Dextran T-2000	$CHCl_3$	0·27
	NaOH	0·38

In contrast, after periodate oxidation of these polymers the solution behaviour, as measured by changes in viscosity and, after periodate oxidation and borohydride reduction by the elution volume on gel chromatography, showed very large differences. Interpretation of results with periodate-oxidised and periodate-oxidised borohydride-reduced polysaccharides is complicated by two effects, the increased susceptibility of the acetal linkage to hydrolysis when the sugar ring is cleaved and a tendency for incomplete oxidation due to acetal formation between newly formed aldehyde groups and existing hydroxyls (Painter & Larsen, 1970).

When a series of (1-4) and (1-6) glycans were oxidised in acetate buffer with periodate and the change in viscosity measured against time of reaction, there was a rapid and substantial decrease for all the glycans. The values ultimately became constant (Table 3 and Fig. 2). The β(1-4) linked structures (galacto- and glucomannans) had the largest decreases followed by α(1-4) and then (1-6). A decrease in viscosity (stiffness) of a number of unbranched polysaccharides such as alginate (Smidsrød & Painter, 1973) amylose and cellulose (Casu *et al.*, 1982, 1985) has been previously observed. In (1-4) and (1-6) linked polymers periodate oxidation splits the pyranose ring and produces an acyclic polymer of 1,3-dioxotrimethylene substituted with hydroxymethyl and C-formyl groups (Casu *et al.*, 1985). The extent of any depolymerisation of these samples was not known but the decrease in viscosity was very rapid and became constant within a reasonable period of time (Fig. 2) consistent with the

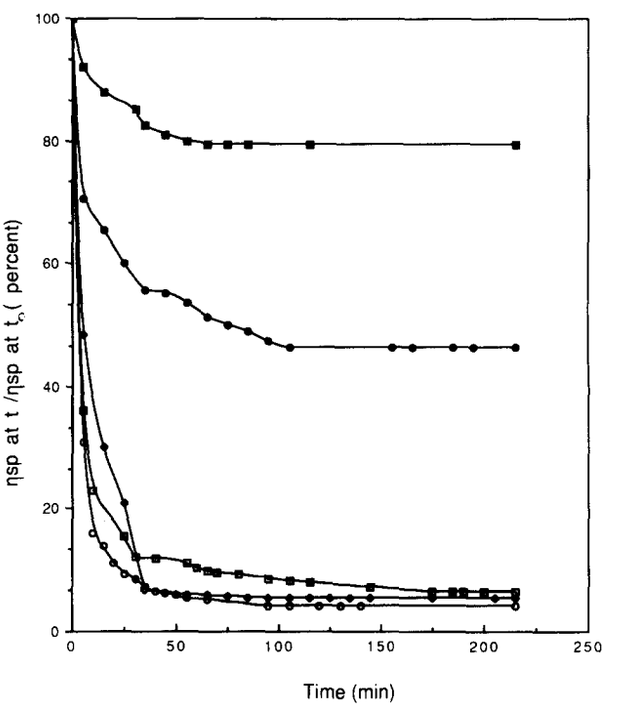

Fig. 2. Viscosity changes of periodate oxidised polysaccharides. (□) konjac glucomannan; (○) locust bean galactomannan; (◇) fenugreek galactomannan; (●) amylose; and (■) dextran T-2000.

Table 3. Viscosity changes on periodate oxidation of polysaccharides

Polysaccharide	Unoxidised (U) or oxidised (O)	Conc. (g/ml)	Time of oxidation[a] (min)	Viscosity number[b] (ml/g)	Final viscosoity/ original viscosity
Fenugreek galactomannan	U	0·117	145	2906	
	O			61	0·02
Locust bean galactomannan	U	0·048	95	3562	
	O			61	0·02
Konjac glucomannan	U	0·037	175	6200	
	O			227	0·04
Amylose	U	0·295	105	414	
	O			34	0·08
Dextran T-2000	U	0·67	65	224	
	O			59	0·26

[a]Time taken for viscosity to become constant.
[b]At concentration shown.

suggestion that the major factor in the decrease was a large conformational change rather than depolymerisation. This and the near completeness of reaction was confirmed by the oxidation of an amylose sample (Fig. 3) when the extent of periodate uptake was compared with the theoretical value and the release of formaldehyde was monitored. Formaldehyde is produced from reducing-end groups and so its rate of release after initial reaction measures the extent of depolymerisation, since any hydrolysis of acetal linkages will produce a source for the production of more formaldehyde. Parrish and Whelan (1959) found that with a series of maltodextrins of known degrees of polymerisation that the extent of overoxidation was sufficiently limited to allow estimation of chain length. Reduction of

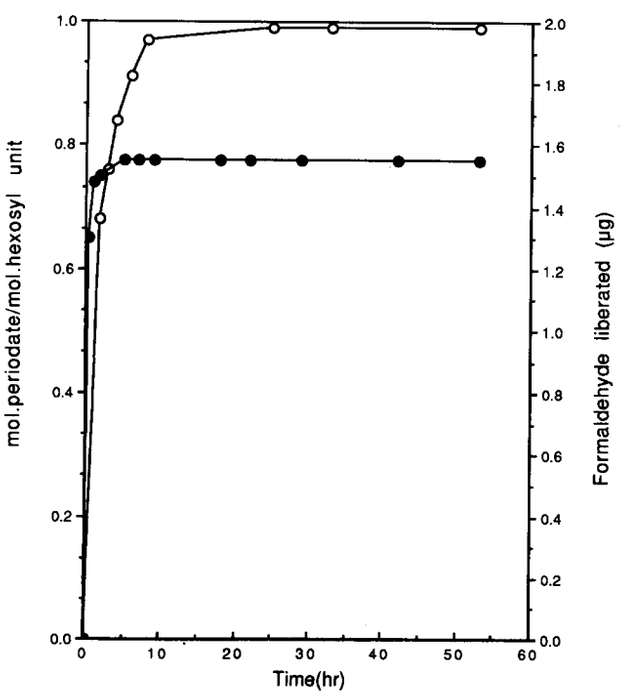

Fig. 3. Periodate uptake and formaldehyde production on oxidation of amylose. (○) mol. periodate/mol. hexosyl unit; and (●) formaldehyde liberated.

periodate was estimated from the change in absorption at 223 nm and formaldehyde production by reaction with chromotropic acid after precipitation of unreacted periodate with lead dithionate (O'Dea & Gibbons, 1953). Oxidation was conducted in unbuffered periodate solution at 4°C in the dark, since these conditions have been reported to cause minimal depolymerisation of oxidised polysaccharides (Parrish & Whelan, 1959; Hough, 1965). The results in Fig. 3 show that periodate uptake stabilised at a value close to theoretical and that following an initial rapid production of formaldehyde in the first 2 h of reaction from the reducing end no signficant increase was detected up to 53 h. It can be concluded that under the reaction conditions depolymerisation was not significant and that uptake of periodate was almost complete. Any hemi-acetal linkages formed during oxidation would be reduced by borohydride and a few remaining isolated unoxidised pyranosyl residues would probably have little effect on the overall shape. Further evidence that depolymerisation was not occurring came from examination by gel chromatography of periodate-oxidised borohydride-reduced amylose during the period of oxidation from 0 to 34 h (Fig. 4). The oxidised-reduced polysaccharide in elution fractions could be sensitively detected from its reaction on heating in acidic solution with 2,4-dinitrophenylhydrazine, followed by reaction with ethanolic alkali. Glycolaldehyde (0–2 µg/ml) provided a suitable standard. Glycerol and erythritol did not react. Phenol-sulphuric acid reagent could be also used but the sensitivity was only one quarter of that with glucose. The $-\log K_{WAV}$ values decreased from 0 to 10 h (similarly to the change of viscosity) but were constant from 10-34 h (Fig. 4), behaviour consistent with an initial major conformational change followed by stabilisation and indicative of the absence of significant depolymerisation.

The elution behaviour of a number of polysaccharides after periodate oxidation and borohydride reduction was then investigated. Oxidation was performed in unbuffered periodate and manipulations were kept to a

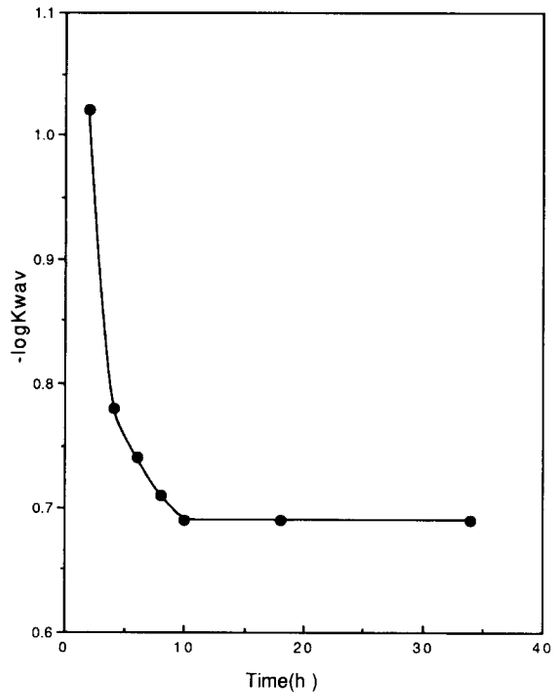

Fig. 4. Change in $-\log K_{WAV}$ values on Fractogel TSK-HW 50 of periodate-oxidised borohydride-reduced amylose with time of oxidation.

minimum. Excess periodate was reacted with dihydroxybutane and iodate removed by centrifugation after the addition of lead acetate. On raising the pH with sodium bicarbonate, borohydride was added immediately and, when reduction was complete, the solution was applied directly to the column of Fractogel.

When konjac glucomannan, fenugreek and locust bean galactomannans, tamarind xyloglucan, amylose and dextran T-2000 were oxidised and reduced the $-\log K_{WAV}$ values of all samples were much lower than those of the original polysaccharide (Table 4). The decrease was more pronounced for the β(1-4) linked polymers than for the dextran and amylose. In two cases (dextran and fenugreek galactomannan) the molecular weight loss due to elimination of formic acid was substantial (30 and 17%, respectively) but even with these the extent of change indicated a major conformational conversion.

The unoxidised polymers can be viewed as repetitively, internally bridged polymers (Fig. 5). Then the (1-4) linked glycans are poly-dioxo-trimethylenes, substituted on one methylene (the C_5 of the pyranosyl ring) with hydroxymethyl or substituted hydroxymethyl (e.g. galactomannan), with a dihydroxy dimethylene bridge (i.e. C_2 and C_3) linking C_1 and C_4. In Fig. 5 the atoms of the dioxo-trimethylene monomer are shown in boldface type and the bridging and other atoms in light type. This bridging completely blocks free rotation about the polymer bonds of four of the five core atoms of a dioxo-trimethylene unit, as well as the two carbons of the bridge. There is partial restriction of rotation about the other oxygen atom (the glycosidic oxygen). This means that in each dioxo-trimethylene monomer, rotation of the polymer bonds of four out of five atoms is completely blocked and the fifth has restricted rotation. Since the ring bonds of the bridging atoms cannot rotate it means six out of seven atoms in the monomer unit are denied rotation and the seventh is partly restricted. It is suggested that this complete blocking of rotation in so many atoms is a major factor in the difference in the hydrodynamic properties of the polysaccharides relative to an acyclic biopolymer such as a peptide chain where rotation is only blocked in a similar way where a proline or (an hydroxyproline) residue occurs. The relationship of polyproline to other polypeptides has analogy to the relationship of polysaccharides to proteins.

After periodate oxidation and borohydride reduction, the monomer unit becomes a substituted dioxo-trimethylene without the bridging and more directly comparable to the amino acid monomers (apart from proline) that

Table 4. The effect of periodate oxidation followed by borohydride reduction on elution on gel chromatography on Fractogel TSK-HW

Polysaccharide	Column matrix	Unoxidised (U) or oxidised (O)	$-\log K_{WAV}$	Calculated MW change (%)
Konjac glucomannan	65(F)	U	0·57	
		O	0·17	+1
Fenugreek galactomannan	65(F)	U	0·60	
		O	0·28	−30
Locust bean galactomannan	65(F)	U	0·80	
		O	0·26	−2
Tamarind xyloglucan	65(F)	U	0·49	
		O	0·15	−6
Amylose	55(S)	U	0·43	
		O	0·26	+1
Dextran T-2000	65(F)	U	0·40	
		O	0·23	−17
Waxy maize starch β-limit dextrin	65(F)	U	V_0	
		O	0·37	0
Glycogen β-limit dextrin	65(F)	U	0·35	
		O	0·34	0

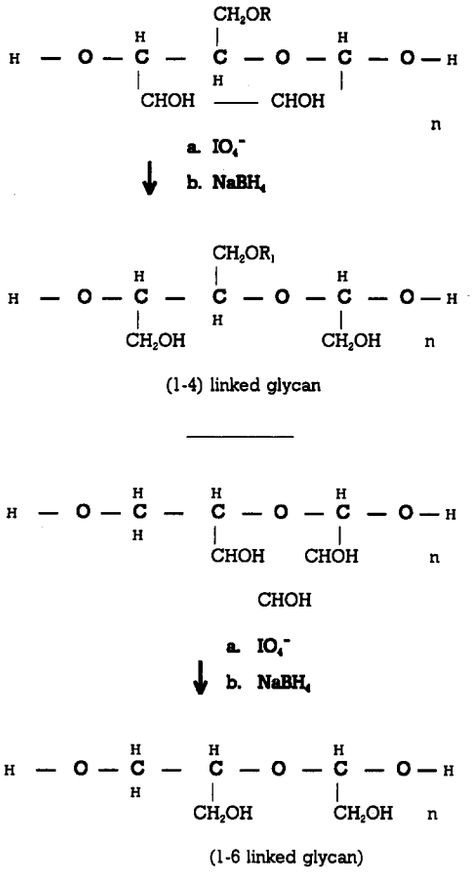

Fig. 5. Comparative structures of glycans and oxidised–reduced derivatives. **(C,O)** carbon or oxygen of dioxo-trimethylene monomer; (C,H,O) side chain atoms; (R), H or glycosyl residues; and (R_1) H or oxidized–reduced glycosyl residues.

form peptides, with respect to the ease of rotation of the bonds of the component atoms of these monomers that are directly involved in the polymer chain.

The decreases in viscosities and $-\log K_{WAV}$ values after periodate oxidation and borohydride reduction are consistent with a change from an extended rod-like shape to a more folded conformation. In the α(1-4) glycan amylose the α-glycosidic linkage leads to a less extended calculated conformation (a tighter helical structure) consistent with the observed smaller relative decrease in viscosity and molecular size on periodate oxidation and borohydride reduction. In a (1-6) polymer (dextran) the comparable change (Fig. 5) in oxidation–reduction is from a polymer with blocked rotation in three atoms out of five of the poly-dioxo-trimethylene chain plus three in a trimethylene bridge, that is two neighbouring atoms in the repeating unit can rotate leading to more flexibility. This gives an even smaller change in viscosity and $-\log K_{WAV}$ values between unoxidised and oxidised compared with the β(1-4) structures. However, there are still significant changes.

The reduction in molecular size associated with the change in conformation resulting from the capacity for rotation in all atoms of the polymer chain in the oxidised–reduced polysaccharides suggested that these derivatives could be characterised by a comparison of their elution rates with standard proteins. With apoferritin (M_W 443 000) and thyroglobulin (M_W 669 000) as standards on Fractogel TSK 65(F), M_r values were obtained for locust bean and fenugreek galactomannans (Table 5). With the additional standard of β-amylase (M_W 200 000) on Fractogel TSK-HW 55(S) values for konjac glucomannen, tamarind xyloglucan, amylose and dextran T-2000 were also obtained. The amylose sample had a lower molecular size prior to oxidation than samples isolated by concanavalin A fractionation of maize starches (Yun & Matheson, 1992), suggesting that selective extraction or depolymerisation may have occurred during isolation and fractionation of this commercial sample. Samples of the β-limit dextrins of rabbit liver glycogen and waxy maize starch were also examined. The $-\log K_{WAV}$ values of these were somewhat higher than that of the standard with the highest

Table 5. Relative molecular weight (M_r) of periodate-oxidised borohydride-reduced polysaccharides from comparison with standard proteins determined by gel chromatography on Fractogel TSK-HW

Source	Matrix	$-\log K_{WAV}$	M_r of periodate oxidised
Locust bean galactomannan	65(F)	0·26	575 000
Fenugreek galactomannan	65(F)	0·28	687 000
Glycogen β-limit dextrin	65(F)	0·34	1 081 000
Waxy maize starch β-limit dextrin	65(F)	0·37	1 356 000
Apoferritin	65(F)	0·23	443 000
Thyroglobulin	65(F)	0·28	669 000
Konjac glucomannan	55(S)	0·50	380 000
Tamarind xyloglucan	55(S)	0·38	247 000
Amylose	55(S)	0·26	135 000
Dextran T-2000	55(S)	0·67	608 000
β-Amylase	55(S)	0·33	200 000[a]
Apoferritin	55(S)	0·58	443 000[a]
Thyroglobulin	55(S)	0·69	669 000[a]

[a]Actual molecular weight.

M_W, but if linearity is assumed for the M_r versus $-\log K_{WAV}$ relationship beyond the extreme standard values then the M_r values shown in Table 5 can be calculated. When these are adjusted for the loss of glucosyl units on β-amylolysis the M_r values calculated for the parent molecules become 2 162 000 for glycogen and 3 014 000 for waxy maize. Estimates of M_W of galactomannans have given a very large range of values, according to the method used (Dea & Morrison, 1975; Tako & Nakamura, 1986). The M_r values obtained here are comparable to those determined by physical methods. Estimates of the M_W of amylopectin and glycogen have led to values in the range of 10^6–10^7.

Glycogen and amylopectin (waxy maize starch) showed a significant difference in the behaviour of the oxidised-reduced limit dextrin compared with the unoxidised (Table 4). For amylopectin β-limit dextrin the $-\log K_{WAV}$ value for the unoxidised was much higher than for the oxidised–reduced product, whereas for glycogen β-limit dextrin the two values were closely similar reflecting the difference in shape believed to exist in the parent polysaccharides—glycogen spherical and amylopectin ellipsoid (Lelievre *et al.*, 1986).

Examination of periodate-oxidised borohydride-reduced derivatives may provide a method for obtaining M_r values of insoluble glycans such as β-mannans, galactoglucomannans and cellulose.

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