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Fructose-grafted amylose and amylopectin

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Abstract

Amylose and amylopectin have been heated with sucrose in acidic methyl sulfoxide. The fructose oxocarbonium ion derived from sucrose was transferred as fructofuranosyl groups to O-6 of the glucans, giving fructoglucans containing up to 7.9% (amylose) and 5.8% (amylopectin) of fructose. The fructosyl units on amylose prevented retrogradation from aqueous solution, resulted in a less-blue iodine complex, and decreased the extent of degradation by amyloglucosidase. The addition of fructosyl units to amylopectin reduced the extent of its degradation by beta-amylase.

Keywords: Fructose-grafted amylose and amylopectin; Amylose; Amylopectin

1. Introduction

Our recent studies of the acid-catalyzed thermolysis of sucrose [1] have shown that the resonance-stabilized fructofuranosyl oxocarbonium ion (**1**) is the first product of such scission of sucrose. This ion has been shown to react with alcohol groups by nucleophilic addition to produce fructosides from simple alcohols [2] and oligosaccharides from unreacted sucrose [1,3]. The kinetic studies with simple alcohols [2] showed that, in methyl sulfoxide solution, primary alcohols react more than an order of magnitude faster than comparable secondary alcohols, and the formation of kestoses [1] confirmed this effect in sucrose melts.

We proposed to extend such studies to systems where **1** is produced in the presence of polysaccharides and of other polymers, in the expectation that fructofuranosyl groups will be transferred from sucrose to the polymers, thereby grafting fructofuranosyl groups onto the polymer backbone. Conditions in this initial study were chosen to achieve this effect without significant degradation of the polymer. Methyl sulfoxide was used as

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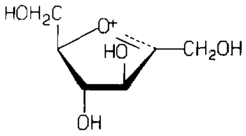
solvent because of previous extensive experience in such systems with sucrose and simple glycosides [2]. Starch components were selected as polymers because of an interest in determining the properties of the fructosyl polysaccharides.



1

2. Results and discussion

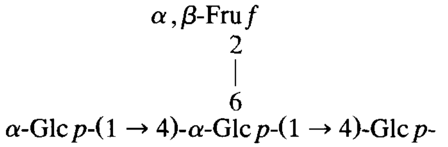
Before any transfructosylation experiments were attempted, it had to be shown that no degradation of amylose occurred under conditions known to induce the thermolysis of sucrose. This was done by measuring the change in the relative viscosity of solutions containing amylose, sucrose, and 10 mM H_2SO_4 in methyl sulfoxide before and after heating for 2 h at 50°C. The relative viscosity before heating was 2.74 and after heating it was 2.71. It was concluded that no significant scission or rearrangement of glucosidic linkages in amylose occurs under these conditions. This fact was confirmed, although less conclusively, by size-exclusion chromatography of the isolated fructosyl amylose, as described later.

Starting with conditions similar to those used in the viscosity determination experiments, the optimization of transfructosylation was performed by varying acidity, temperature, time, and reactant ratios and determining the fructose-to-glucose ratios in the resulting fructosyl amylose products by combined cysteine–carbazole and sulfuric acid colorimetric analyses. Results are shown in Table 1. Conditions of 50°C/2 h/sucrose:amylose (5:1 w/w)/10mM H_2SO_4 were chosen for convenience and efficiency and on a large scale gave a fructosyl amylose product containing 4.6% fructose (i.e., ca. 1 fructose residue:22 glucose residues).

Table 1
Fructosylation of amylose

Conditions					
Temperature (°C)	Time (H)	Sucrose:amylose	H_2SO_4 mM	Yields % based on amylose	% Fructose
70	1	1:1	10	98	1.6
70	1	2:1	10	98	2.9
70	1	5:1	10	96	4.2
85	0.5	5:1	10	94	3.2
55	3	5:1	10	103	6.4
42	9	5:1	10	106	7.6
25	65.5	5:1	10	102	7.9
42	4.5	5:1	20	106	6.7

The fructosyl amylose polymer was analyzed using conventional methylation analysis and compared with amylose. In order to detect the presence of fructose residues, which are very subject to acid degradation in the hydrolysis phase of methylation analysis, a double procedure using mild and strong hydrolysis was performed on duplicate samples of the fructosyl amylose as described earlier [4]. GC–MS from the mild hydrolysis procedure showed only 2,5-di-*O*-acetyl-1,3,4,6-tetra-*O*-methylhexitol(s) resulting from fructofuranosyl grafted units. The strong hydrolysis procedure, followed by GC–MS and GC–FID (corrected for carbon response [5]) showed products from glucopyranose units which were 1,4 – linked-(1.00), 2,3,6-linked (0.048), and 1-linked (0.021). The 2,3,6-linked glucose units were not detected from methylation analysis of the original amylose, therefore they must arise from new branch points at which fructose units are grafted at O-6 of in-chain glucose units of the amylose. The relative amount of such units from the methylation analysis corresponds to 4.5% of the product polysaccharide and agrees well with the fructose content of 4.6% from colorimetric determination of the fructose:glucose ratio. The grafted fructose units are evidently in the furanose form as shown by the identity of the derived tetramethylhexitol(s). The grafting occurs only at O-6 of the amylose as expected from earlier studies with primary and secondary alcohols [2]. There is no further addition of fructose cation (**1**) to fructose units already grafted to the amylose (i.e., all of the fructose is present as single unit side chains). By analogy with previous results [1,2], we assume that the fructofuranosyl groups are both α- and β-linked and that the general structure is as shown in **2**.



$$\alpha\text{-Glc}p\text{-}(1\rightarrow 4)\text{-}\alpha\text{-}\overset{\overset{\alpha,\beta\text{-Fru}f}{\overset{2}{\overset{|}{6}}}}{\text{Glc}}p\text{-}(1\rightarrow 4)\text{-Glc}p\text{-}$$

2

The fructose groups appended to the amylose chain at average intervals of ca. 20 glucose units were expected to interfere with formation of extended sequences of the stable amylose helix, which contains about six glucose units per turn. This question was addressed by study of the iodine complex. The complex of I_2/KI with amylose had $\lambda_{\max}$634 nm, whereas the fructosyl amylose had $\lambda_{\max}$584 nm, with a similar extinction coefficient. This shift in $\lambda_{\max}$, showing a complex that is less blue, indicates a partial transition towards a red iodine complex similar to that shown by amylopectin and which is generally associated with disruption or inhibition of the formation of extended helixes by branches in the amylopectin chain [6].

The retrogradation of amylose from aqueous solution is generally considered [7] to be due to alignment and hydrogen-bonding of molecules to form ''bundles'' and eventually insoluble particles. The presence of fructose units along the amylose chain was expected to inhibit such alignment and bonding along the chains and hence to decrease retrogradation. Accordingly we found that, under conditions where a neutral amylose solution showed 75% precipitation due to retrogradation in 24 h, the corresponding fructosyl amylose showed no precipitation.

The hydrolysis of starches to glucose by amyloglucosidase is generally complete because this enzyme attacks both α-(1 → 4) and α-(1 → 6) glucosyl linkages [8]. It was anticipated however that the presence of a fructofuranosyl group at intervals along the amylose backbone would stop the progressive action of this exo-enzyme. Accordingly we found that under conditions where amylose was 86% converted into apparent glucose (as measured by reducing power) the fructosyl amylose was only 29% converted. At this stage, the hydrolysis of the fructosyl amylose was still proceeding at a significant rate however, and it is possible that the alpha-glucosidase can slowly attack the fructofuranosyl linkages. The digests at 29% conversion showed glucose and a trace of fructose, but no oligosaccharides. This system would merit further study.

With alpha-amylase, starches are attacked in endo-fashion, although there is some resistance to hydrolysis at or near branch points in amylopectin [9]. The action of alpha-amylase on fructosyl amylose (as indicated by increase in reducing power) was only slightly less extensive than with amylose. Thus there was an apparent conversion to glucose (by reducing power) of 52% with amylose and 43% with the fructosyl amylose in 300 min. LC of the latter products at this stage, suggested the presence of oligosaccharides containing about 5–7 hexose units, but with slightly different retention times from the corresponding maltooligosaccharides. Presumably these contain a fructose component, but they were not further investigated. We conclude that the alpha-amylase probably does not readily attack the fructofuranosyl linkages in fructosyl amylose.

Amylopectin was also treated with sucrose and H_2SO_4 in the same way as with amylose. The resulting fructosyl amylopectin (obtained in 104% yield based on amylopectin) had 5.8% fructose by colorimetric analysis. As it had already been shown that amylose suffers no glucosidic scission or rearrangement under these conditions, it was assumed that the original amylopectin structure also remained intact. Size-exclusion chromatography confirmed that the fructosyl amylopectin had a molecular weight above 4×10^5 (pullulan standard). Methylation analysis was carried out using both mild and strong hydrolysis [4] and compared with the original amylopectin. The mild hydrolysis showed that the fructose was present only as non-reducing fructofuranosyl end-groups. The strong hydrolysis showed by GC–MS that only (1 → 6)-branch points were present both before and after fructosylation. The GC–FID quantitation (corrected for e.c.r. [4]) showed that in the original amylopectin there were 14 unbranched in-chain glucose units for each branched glucose unit. In the fructosyl amylopectin, the same analysis showed eight unbranched in-chain glucose units for each branched glucose unit. We conclude that the fructosyl amylopectin contains about as many fructose-branched in-chain glucose units as "normal" branched units. This value requires a fructose content of 5.3% if all of the fructose branches are single fructose units, and we conclude that this is indeed the case, since the colorimetric analysis indicates 5.8% fructose content and since no non-end-group fructose groups were detected. The fructosyl amylopectin therefore carries single fructofuranosyl groups at O-6, about equal in number to the original (1 → 6)-branch points of the amylopectin.

The action of the exo enzyme beta-amylase on the fructosyl amylopectin was compared with the original amylopectin. The conversion limit was reached in the same time in both instances, with the original amylopectin showing 66.3% conversion into apparent maltose (by colorimetry) and the fructosyl amylopectin showing 31.7% conversion. These values are compatible with the foregoing concept of the fructosyl amylopectin structure.

Future studies will deal with the development of solvent-less conditions for thermal transfructosylation.

3. Experimental

Materials.—Amylose type III from potato, amylopectin from corn, amyloglucosidase [exo-(1 → 4)-α-D-glucan glycohydrolase, EC 3.2.1.3] from *Aspergillus niger*, alpha-amylase [(1 → 4)-α-D-glucan glucanohydrolase, EC 3.2.1.1] type IIA from *Bacillus sp.*, and beta-amylase [(1 → 4)-α-D-glucan maltohydrolase, EC 3.2.1.2] type IB from sweet potato were obtained from Sigma Chemical Company.

General methods.—Fructose:glucose ratios in the fructoglucans were determined by a combination of the cysteine–carbazole–H_2SO_4 and phenol–H_2SO_4 methods as described earlier [4]. Methylation analysis utilized both mild and strong hydrolysis conditions on separate samples, followed by reduction with sodium borodeuteride, GC–MS and GC–FID as described earlier [4]. Reducing power determinations in enzyme experiments were carried out with alkaline 3,5-dinitrosalicylate [10], using either glucose or maltose references.

Investigation of amylose degradation.—Dry amylose (0.804 g) was pasted with dry methyl sulfoxide (16 mL) then heated in a boiling-water bath for 20 min to complete dissolution. Dry powdered sucrose (4.00 g) was added, the mixture sonicated to dissolve at room temperature and then 50 mM H_2SO_4 in methyl sulfoxide (4 mL) was added. This solution was heated in a water-bath at 50 ± 1°C. At zero time and after 2 h of heating, a sample (5.0 mL) was diluted with methyl sulfoxide (15 mL) and the viscosity determined in a capillary viscometer. The relative viscosity was 2.74 at zero time and 2.71 at 2 h.

Preparation of fructosyl amyloses.—Several methyl sulfoxide solutions (20 mL each) containing various proportions of amylose, sucrose, and H_2SO_4 were prepared exactly as above, then heated for varying periods at various temperatures as shown in Table 1. After cooling, the solution was neutralized with 0.04 M KOH followed by water (30 mL) and then abs EtOH (200 mL) was added dropwise with stirring to produce a fine white precipitate. This was recovered by centrifugation, washed twice with 95% EtOH, then with abs EtOH and acetone and dried at ca. 40°C in vacuo to constant weight. Yields and fructose contents are shown in Table 1. LC examination of the products [1] showed no detectable mono-, di-, or oligo-saccharides, which were removed in the supernatant phase of the precipitation.

Retrogradation of amylose and fructosyl amylose.—Amylose (0.10 g) and fructosyl amylose (0.10 g) were separately pasted with 1 M NaOH (1.00 mL) and sonicated for 15 min to dissolve them. Water (7.0 mL) was added to each solution, followed by 1 M AcOH (1.10 mL) added with stirring (pH 5.5). Water was added to a total solution weight of 10.2 g and the solutions were kept at 0°C. At intervals, samples (0.10 mL) were withdrawn, diluted to 10 mL, centrifuged, and the centrifugate used for phenol–H_2SO_4 assay of total dissolved carbohydrate, using glucose reference solutions. With amylose, 75% of the originally dissolved polymer had precipitated in 24 h. With the fructosyl amylose (4.6% fructose), no precipitation was observed and the glucose analysis of the solution remained unchanged up to 30 h.

Iodine complexation with fructosyl amylose.—Amylose (5.1 mg) and fructosyl amylose (5.1 mg, 4.6% fructose) were separately dissolved in 0.5 M NaOH (1.0 mL), briefly (3 min) heated in a boiling-water bath to complete the dissolution, cooled and 0.5 M HCl (1.0 mL) was added. Water (400 mL) and potassium hydrogen tartrate (0.96 g) were added, followed by a solution of KI (100 mg) and I_2 (10 mg) in water (5 mL) and finally the solutions were diluted to 500 mL. After ca. 20 min at room temperature the spectrum from 500–800 nm was measured against the appropriate KI/I_2 blank. The amylose showed absorbance 0.181 (10 mm pathlength) at λ_{max} 633 nm, while the fructosyl amylose showed 0.192 at λ_{max} 584 nm.

Action of amyloglucosidase on fructosyl amylose.—Amylose (51 mg) and fructosyl amylose (52 mg, 4.6% fructose) were separately dissolved in 1.0 M NaOH (0.5 mL), diluted with water, neutralized with 0.1 M AcOH to pH 4.5 then made up to 50 mL. These solutions were equilibrated in a water-bath at 55°C before addition of the enzyme solution (20 μL) containing 10 mg/mL protein and 64 units/mg. At intervals samples were withdrawn for determination of reducing power with alkaline 3,5-dinitrosalicylate using glucose reference. After 5 h the amylose yielded 86% and the fructosyl amylose 29% apparent glucose.

Action of alpha-amylase on fructosyl amylose.—In a similar experiment to that just described, using pH 6.9, 25°C and an alpha-amylase solution (20 μL) containing 14 units, the amylose showed 52% and the fructosyl amylose 43% conversion to apparent glucose in 5 h.

Preparation of fructosyl amylopectin.—The reaction and isolation were carried out exactly as already described for amylose, using 5:1 (w/w) sucrose:amylopectin and 10 mM H_2SO_4 for 2 h at 50°C. The fructosyl amylopectin was obtained in 104% yield based on amylopectin and contained 5.8% fructose.

Action of beta-amylase on fructosyl amylopectin.—Amylopectin (0.125 g) and the fructosyl derivative (0.125 g) were separately dissolved in 0.1 M pH 5 sodium acetate buffer (25 mL) with heating in a boiling-water bath for 5 min to complete solution. The solutions were incubated at 30°C with the enzyme (111 units) and at intervals samples were withdrawn for reducing-power analysis with alkaline 3,5-dinitrosalicylate reagent using maltose as reference.

Acknowledgements

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References

[1] M. Manley-Harris and G.N. Richards, *Carbohydr. Res.*, 219 (1991) 101–113, and references therein.
[2] W. Moody and G.N. Richards, *Carbohydr. Res.*, 97 (1981) 247–255.
[3] M. Manley-Harris and G.N. Richards, *Carbohydr. Res.*, 154 (1994) 195–202.
[4] M. Manley-Harris and G.N. Richards, *Carbohydr. Res.*, 240 (1993) 183–196.
[5] D.P. Sweet, R.H. Shapiro, and P. Albersheim, *Carbohydr. Res.*, 40 (1975) 217–225.
[6] C.T. Greenwood, in W. Pigman and D. Horton (Eds.), *The Carbohydrates*, Academic Press, NY, 1970, p 494.
[7] C.T. Greenwood, in W. Pigman and D. Horton (Eds.), *The Carbohydrates*, Academic Press, NY, 1970, p 492.
[8] N.K. Matheson and B.V. McClearey, in G.O. Aspinall (Ed.), *The Polysaccharides*, Academic Press, NY, Vol. 3, 1985, p 70.
[9] N.K. Matheson and B.V. McClearey, in G.O. Aspinall (Ed.), *The Polysaccharides*, Academic Press, NY, Vol. 3, 1985, pp 35–37.
[10] W.J. Whelan, *Methods Carbohydr. Chem.*, (1964) 256.