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Carbohydrate Research 278 (1995) 363–366

CARBOHYDRATE RESEARCH

Note

Thermal transfer of fructosyl residues to amylopectin and soluble starch during the melt thermolysis of sucrose

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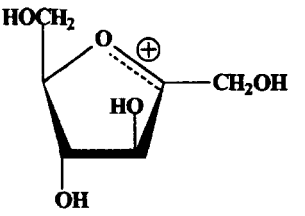
Received 1 May 1995; accepted 4 July 1995

Keywords: Thermal transfer; Fructosyl; Amylopectin; Soluble starch; Thermolysis

During the acid-catalyzed melt thermolysis of sucrose, fructosyl cation (**1**) and glucose are formed. Cation **1** may react with hydroxyl groups on sucrose or glucose, or on other material included in the melt.



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We have used this reaction to form kestoses (fructosylsucroses) [1] and a fructoglucan polymer [2]. Using cyclodextrins as a model, we have demonstrated that fructosyl residues can be transferred to starch-like molecules included in the melt [3]. We have previously demonstrated the formation of fructosylamylopectin (Fru-AP) and fructosylamylose in solution in Me_2SO [4] and we now report the formation of Fru-AP and

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SSDI 0008-6215(95)00252-9

fructosyl-soluble starch (Fru-SS) by thermal transfer of fructosyl residues from sucrose in the melt.

An intimate, amorphous mixture of amylopectin and a five-fold excess of sucrose acidified with citric acid was obtained by freeze-drying. Portions of this mixture were thermolyzed in open containers. The product, Fru-AP, was precipitated twice from water with ethanol and examined by GPC using Bio-Gel P-2. The sample was completely excluded by this gel, which indicated that all monosaccharides and oligosaccharides of dp $< \sim 20$ had been removed by the precipitation. A similar treatment of soluble starch yielded Fru-SS.

Colorimetric assay of the Fru-AP product indicated a fructose content of 12%. Mild hydrolysis followed by reduction and per-*O*-trimethylsilylation using an internal standard indicated an 8% content of fructose. For steric reasons, **1** would be expected to add predominantly to primary hydroxyl groups, that is, to O-6 of glucose. Addition of fructose would therefore be expected to result in an increased number of apparent 1,4,6-branch points. Methylation analysis indeed revealed an increase in the number of these branch points from 7%, that is ~ 14 unbranched in-chain glucose residues for every branched residue, in amylopectin to 17% in Fru-AP, that is ~ 5 unbranched in-chain glucose residues for every branched glucose residue; no evidence was found of other than 1,4,6-branch points. However, methylation analysis gives no indication of the presence of fructose attached to other fructose units since the strong hydrolysis conditions required to cleave the amylopectin chain completely destroy the fructose residues. Mild hydrolysis of per-*O*-methylated Fru-AP revealed only 2-linked fructofuranose units, indicating that fructose residues which have been further substituted, if they do exist, constitute an insignificant proportion of the total fructose.

A further confirmation of an increased degree of branching was obtained by the enzyme studies described next.

Colorimetric assay of Fru-SS indicated 12% fructose. Mild hydrolysis followed by reduction and per-*O*-trimethylsilylation revealed, by GC-FID, 14% of fructose. Methylation analysis showed an increase in branched residues from 4% in untreated soluble starch to 14% in Fru-SS. The latter value is not a measure of the total fructose present, since mild hydrolysis of the per-*O*-methylated Fru-SS showed that $\sim 20\%$ of the fructose residues were 1,2- or 2,6-linked, that is to say, chains of two or more fructose units were attached at some branch points. The ^{1}H NMR spectrum of Fru-SS showed signals corresponding to H-3 and H-4 of fructose [3] and the ^{13}C NMR spectrum contained small signals at 108.9 and 104.8 ppm, indicating the presence of both α- and β-linked fructofuranosyl residues [1].

Enzyme studies.—The effect of three enzymes, beta-amylase, alpha-amylase, and amyloglucosidase, was studied. The reaction was monitored by reducing-end-group analysis using 3,5-dinitrosalicylate [5]. This assay can be regarded as reasonably quantitative for the maltose and glucose produced by beta-amylase and amyloglucosidase respectively, but the result for alpha-amylase should be regarded as only a qualitative indication of the extent of enzyme activity. The assay has been shown [6] to overestimate enzyme activity when used to determine reducing values in mixtures of oligosaccharides of differing chain-lengths greater than dp 2.

The beta-amylase limit for amylopectin was 55% conversion into apparent maltose

whereas the limit for Fru-AP was 9%. The exo-acting beta-amylase is therefore unable to degrade the polymer past a branch-point, whether this is a glucosyl or a fructosyl residue attached at C-6. The beta-amylase limit for soluble starch was 64% conversion, whereas that for the Fru-SS was 0%. This rather startling observation was confirmed by repeating the experiment twice, the second time being with a ten-fold increase in the concentration of the enzyme. The Fru-SS bears on average a fructosyl residue on every eighth residue, and it would appear from these results that the fructosyl branches are partly concentrated near the non-reducing ends of the soluble starch molecules.

Reaction with porcine pancreatic alpha-amylase, an endo-acting enzyme, gave, after 20 h, 70 and 34% conversion into apparent maltose, respectively, for amylopectin and Fru-AP. The corresponding values for soluble starch and Fru-SS were 59 and 8%.

Amyloglucosidase is an exo-acting enzyme capable of hydrolyzing (1 → 4)-α-D- and, albeit more slowly, (1 → 6)-α-D-linkages. This enzyme gave, after 20 h, 52 and 15% conversion to apparent glucose, respectively, for amylopectin and Fru-AP. The corresponding values for soluble starch and Fru-SS were 95 and 21%. The action of this enzyme is less restricted by fructofuranosyl branches than is beta-amylase.

1. Experimental

General methods.—Methylation analyses were performed with NaOH and MeI in Me_2SO [7] with modifications described in ref. [8]. Colorimetric assays for fructose were carried out using the cysteine–carbazole test [9]. Mild hydrolyses of Fru-AP and Fru-SS were effected in HOAc (1 M) for 90 min at 90°C with an internal standard of xylitol; samples were blown dry at < 40°C, reduced with $NaBD_4$ in 1 M NH_4OH (3 h, 25°C), and per-*O*-trimethylsilylated for subsequent assay by GC-FID using a fused-silica capillary column (Hewlett–Packard Ultra 2, 25 m × 0.33 m) packed with crosslinked phenyl methyl silicone; conditions used were 55°C for 1 min, 30°C/min to 220°C, and 10/min to 320°C. Gel chromatography was effected using a 2.5 cm × 90 cm column packed with Bio-Gel P-2 eluted with water at 0.5 mL/min; detection by RI. Reducing-end-group analyses during the enzyme assays were achieved using alkaline 3,5-dinitrosalicylate [5]. NMR spectra were measured on a Varian Unity 400-plus spectrometer in D_2O and chemical shifts are recorded relative to external Me_4Si.

Preparation of Fru-AP and Fru-SS.—Amylopectin (10.25 g, moisture content 10.3%, Sigma Chemical Co. from corn), sucrose (50.0 g), and citric acid (0.60 g) were dissolved in water (450 mL) and freeze-dried to yield a brittle, white, solid foam. A sample of this product began to melt at 89°C and became a clear, colorless liquid by 104°C. A further sample (1.01 g) was crushed to a thin layer in a 50 mL Erlenmeyer flask and heated in an oven at 140° ± 1°C for 30 min. The resultant pale-yellow melt was cooled, dissolved in water, neutralized with NH_4OH and the product precipitated with EtOH (100 mL). The precipitate was redissolved in water and reprecipitated, and then washed with EtOH and dried to a white powder (0.150 g) described herein as Fru-AP. Soluble starch (10.0 g, moisture content 11.5%, Sigma Chemical Co. A.C.S. reagent), sucrose (50.0 g) and citric acid (0.60 g) were dissolved in water (250 mL) and freeze-dried. A sample (2.00 g) of the product was heated as before for 100 min at

120°C, precipitated twice from water with EtOH, and dried to a white powder (0.32 g) described herein as Fru-SS.

Action of beta-amylase.—Substrates (0.05 g) were dissolved in NaOAc buffer (pH 4.8, 25 mL, 0.1 N) with boiling. The solutions were cooled and incubated at 23°C with 10 μL of a solution containing beta-amylase (11 units, Sigma, EC 3.2.1.2, Type I-B from sweet potato). Samples were withdrawn at intervals and assayed for maltose by reducing-end-group analysis.

Action of porcine pancreatic alpha-amylase.—Substrates (0.05 g) were dissolved in citrate–phosphate buffer (pH 6.9, 25 mL) with boiling. The solutions were cooled and incubated at 20°C with 10 μL of a solution containing porcine pancreatic alpha-amylase (12 units, Sigma, EC 3.2.1.1, Type I-B DFPT-treated). Samples were withdrawn at intervals and assayed for maltose by reducing-end-group analysis.

Action of amyloglucosidase.—Substrates (0.05 g) were dissolved in NaOAc buffer (pH 4.5, 25 mL, 0.1 N) with boiling. The solutions were cooled and incubated at 55°C with 200 μL of a solution containing amyloglucosidase (10.8 units, Sigma, EC 3.2.1.3 from *Rhizopus* genus mold). Samples were withdrawn at intervals and assayed for glucose by reducing-end-group analysis.

Acknowledgements

The authors thank Mr Cody Dwire for experimental assistance. This work was supported by a grant from The Sugar Association, Inc.

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