The Codextrinization of Amylopectin with Radioactive Glucose and Partially Methylated Glucose[1]

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ABSTRACT

Dextrinization of amylopectin in the presence of uniformly labeled radioactive D-glucose at a mole ratio of 6,000:1 resulted in a product containing 72% of the labeled glucose. Similar reactions of amylopectin with 2,3,6-tri-O-methyl-D-glucose at mole ratios of 2:1 and 6:1 yielded dextrins with 3.6 and 0.9% of the methylated sugars respectively.

During the process of dextrinizing polysaccharides, glycosidic linkages are cleaved and new acetal linkages are formed with the resulting polysaccharide having a rearranged structure and additional branching (1). It is reasonable to believe that monomeric molecules containing groups capable of forming acetals might be incorporated into the product of dextrinization if present during the reaction. Indeed, Christensen (2) has prepared a number of dextrins containing monosaccharide molecules not present in the original polysaccharide by heating a mixture of polysaccharide and a pentose or hexose in the presence of an acid catalyst.

There are those workers who doubt this claim, however, and express the opinion that the presence of foreign sugars in the dextrin hydrolysates result from acid reversion with the formation of new polysaccharides containing only the added monosaccharides. Inadequate purification of the dextrins is thought to leave a mixture of a dextrin derived from the polysaccharide and a polymer generated from the added sugar.

In an effort to obtain additional information on this subject, amylopectin was dextrinized under acidic conditions with uniformly labeled radioactive glucose and also with 2,3,6-tri-*O*-methyl-D-glucose.

MATERIALS AND METHODS

Dextrinization of Amylopectin with [U-^{14}C]-Glucose

D-[U-^{14}C]-Glucose (0.375 mg.) in a water-methanol solution (1 ml. of 25% methanol) was added to defatted corn amylopectin (2 g.). The specific activity of the uniformly labeled glucose was 40 μc./mg. Additional water (1 ml.) was added which produced a paste and afforded efficient distribution of the glucose throughout the amylopectin. The solvent was removed under reduced pressure and the dry powder was sprayed with 2.2*N* hydrochloric acid (0.08 ml.). After remaining overnight at room temperature the mixture was heated at 140°C for 3 hr. with stirring in a nitrogen atmosphere. The product was dissolved in water (12 ml.) and a small resi-

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due (0.08 g.) was removed by centrifugation. An aliquot (6 μl.) was spotted on a disk of Whatman No. 1 filter paper. The remaining portion of the solution was added dropwise to absolute ethanol with stirring, and the precipitated dextrin was washed with ethanol. The product was redissolved and reprecipitated twice more in the same manner before being washed with ethyl ether and petroleum-ether and dried *in vacuo*. The product (1.50 g.) was dissolved in water (12 ml.) and an aliquot (6 μl.) spotted similarly on another paper disk.

The filter paper disks were counted for beta radiation. The sample from the reaction mixture gave 1,609 and that from the purified dextrin, 1,165 counts/min. after correction for the background count (23 counts/min.).

An aliquot (6 μl.) of the purified dextrin solution was chromatographed on Whatman No. 1 paper with pyridine-ethyl acetate-water (1:2.5:3.5, upper phase) as the irrigation solvent and ammoniacal silver nitrate (3) as the spray reagent. No glucose was detected on the chromatogram.

Codextrinization of Amylopectin and 2,3,6,-Tri-O-Methyl-D-Glucose

(a) *Dextrinization 1.* Defatted amylopectin (2.1 g., 0.013 moles; a mole of starch is intended to mean the gram formula weight of one anhydroglucose unit) was treated with 2,3,6-tri-*O*-methyl-D-glucose (1.5 g., 0.0065 moles) in methanol (5 ml.). The materials were mixed efficiently and the methanol removed at reduced pressure. The mixture was sprayed with 2.2*N* hydrochloric acid (0.15 ml.), kept at room temperature overnight, and dextrinized as described in the preceding experiment. The reaction mixture was treated with water (15 ml.) and, after removal of a trace of insoluble material, the codextrin was precipitated by slowly pouring the solution into absolute ethanol with stirring. The precipitate was redissolved and reprecipitated twice more in the same manner before being washed with ethyl ether and petroleum-ether and dried *in vacuo*. The product (1.4 g.), $[\alpha]^{28}_{D}$ + 151° in water, (*c*, 1.2), had a methoxyl value of 1.62% which is equivalent to 3.6% of 2,3,6-tri-*O*-methyl-D-glucose by weight in the codextrin. A blank methoxyl determination was performed on the amylopectin (found: OCH_3, 0.00%).

A portion of the product (0.5 g.) was hydrolyzed with *N* sulfuric acid (10 ml.) for 10 hr. on a boiling water-bath. The solution was neutralized with Duolite A-4 anion exchange resin and then concentrated to a syrup. Paper-chromatographic analysis of the syrup with butanone-water azeotrope and spray reagent *p*-anisidine trichloroacetic acid (4) revealed the presence of 2,3,6-tri-*O*-methyl-D-glucose and D-glucose in the hydrolysate.

(b) *Dextrinization 2.* The unreacted methylated sugar (1.17 g., 0.005 mole) was recovered from the washings of the previous experiment and dissolved in acetone (5 ml.). The solution was added to defatted amylopectin (5 g., 0.03 mole) and after thorough mixing the solvent was evaporated. The powdery residue was sprayed with 2.2*N* hydrochloric acid (0.26 ml.) and dextrinized at 140°C. for 3 hr. after standing overnight at room temperature. The reaction was performed at reduced pressure (water pump) with frequent shaking to prevent localized heating. An aqueous solution of

the product was precipitated with ethanol and after further reprecipitations the product was extracted with acetone for 12 hr., with a Soxhlet apparatus. The amorphous, tan material (3.1 g.) showed $[\alpha]^{28}_{D}$ + 146° in water (*c*, 1.0) and a methoxyl value of 0.39%. This value corresponded to a 0.85% methylated sugar content by weight. The acetone extract gave no methylated sugar on evaporation to dryness.

The purified dextrin was hydrolyzed as described in the previous experiment and 2,3,6-tri-*O*-methyl-D-glucose and glucose were identified chromatographically.

DISCUSSION

The dextrinization of amylopectin in the presence of uniformly labeled radioactive glucose at a molar ratio of 6,000 to 1 resulted in a 72% incorporation of the labeled glucose into the dextrin. No free glucose was chromatographically detectable in the product.

Further evidence to support the concept of codextrinization was obtained from the acid-catalyzed dextrinization of amylopectin in the presence of 2,3,6-tri-*O*-methyl-D-glucose again at an elevated temperature. The products were isolated and thoroughly extracted with solvents which would remove any methylated sugar or polysaccharide resulting from acid reversion of this compound. Methoxyl determinations on the purified products showed that 3.6% of the methylated sugar had been incorporated into the dextrin when the mole ratio of amylopectin to 2,3,6-tri-*O*-methyl-D-glucose was 2:1. An incorporation of 0.9% was obtained when the respective ratio was 6:1. Hydrolysis followed by chromatographic analysis of both codextrins established the presence of the methylated sugar. The small amount of the new monomer unit in each product may be accounted for on the basis of steric hindrance provided by the methyl groups and the relative inactivity of the C_4 hydroxyl.

The results of these experiments strongly support codextrinization and the incorporation of new monomeric units into a dextrinized polysaccharide. Certainly the probability of a radioactive glucose molecule reacting with the polysaccharide far exceeds the likelihood of its reacting with another glucose molecule at the mole ratio employed in this experiment. The presence of methylated glucose in the hydrolysate of the codextrin after purification which would remove this material or a polymer derived from it by acid reversion offers additional confirmatory evidence.

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