

ISOLATION OF MALTULOSE FROM ALPHA-AMYLASE HYDROLYSATES OF WAXY CORN STARCH¹

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ABSTRACT

The products of exhaustive salivary alpha-amylolysis of defatted waxy corn starch have been examined for anomalous sugars. Maltulose (4-*O*-alpha-D-glucopyranosyl-D-fructose) was isolated in low yield, indicating the occurrence of D-fructose in waxy corn starch. No free nigerose was liberated by the enzyme.

Extensive investigations on starch have established fairly well its main structural features. Recent efforts have been concentrated on evaluating the significance of minor amounts of anomalous linkages which appear to be present in the starch molecule. The existence of linkages other than the alpha- (1→4)- and alpha- (1→6)- has recently been reported (12,18). The possibility that these are artifacts caused by acid reversion (11) or acid-catalyzed transglucosylation (transglucosidation) (9) cannot be excluded.

Selective hydrolysis of the linkages in starch by specific enzymes would eliminate the uncertainties of acid-catalysis. The present work describes the use of salivary alpha-amylase to hydrolyze the alpha- (1→4)-linkages in waxy corn starch, and examination of the end-products for anomalous sugars.

Material and Methods

Salivary Alpha-Amylase. Human saliva was filtered through a layer

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of diatomaceous earth and stored under toluene at 2°C. It showed no maltase activity when incubated with a maltose solution for 48 hours.

Analytical Methods. Reducing power was determined by the ferricyanide-ceric sulfate method of Hassid (5) and total carbohydrate by the anthrone method of Haas and Fleischman (4). Fructose was determined by the alcoholic anthrone method of Wise and co-workers (17). The micromodification of the Willstätter-Schudel method of hypiodite oxidation was used (1).

Qualitative analysis of fractions by paper chromatography was done by a single development of 48 hours on Whatman No. 3 MM paper. Irrigation solvent was n-butanol-ethanol-water, 9:5:4 (13). Presence of sugars on papergrams was shown by reaction to aniline-diphenylamine reagent, of aldoses by aniline phthalate and benzidine, and of ketoses by alpha-naphthol and orcinol (7).

Zone electrophoresis was carried out on Reeve-Angel glass fiber filter paper on 0.5M sodium tetraborate (2), on apparatus similar to that of Kunkel and Tiselius (8).

Hydrolysis of Waxy Corn Starch. Ninety grams of defatted waxy corn starch were boiled for 30 minutes to a thin paste in 3 l. of distilled water. Filtered saliva (10 ml.) and some toluene were added to the cooled mixture (pH 6.4) and this was incubated at 37°C. The limit of hydrolysis was reached in 48 hours and could not be extended by a further addition of 5 ml. of saliva. Hydrolysis was stopped by bringing the mixture quickly to a boil and then cooling. The hydrolysate, after being filtered to remove a slight precipitate, was concentrated to 200 ml. A control of 1.5% maltose solution plus saliva was similarly treated.

Fractionation of Products. The di- and trisaccharide fractions of the concentrate were separated on a column (50×800 mm.) of Darco G-60-Celite by the technique of Whistler and Durso (14). These were concentrated and analyzed by paper chromatography.

The disaccharide portion was further fractionated on large sheets of heavy filter paper (Whatman No. 3 MM) with the n-butanol-ethanol-water mixture as the irrigating solvent.

Results and Discussion

The only catalytic function salivary alpha-amylase is known to perform is the hydrolysis of the alpha-(1→4)-linkage. Alpha-(1→6)-linkages and any others would presumably remain intact.

Hydrolysis ceased after 48 hours at 69% conversion to maltose. Examination of the digest by paper chromatography revealed 48% maltose, 32% maltotriose, and 20% limit dextrins as the products.

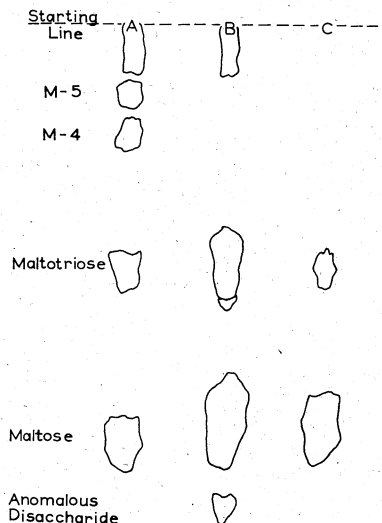


Fig. 1. Paper chromatographic fractionation of end-products; A, reference sugars; B, enzymatic hydrolysate of waxy corn starch; C, enzyme-maltose control.

Another sugar was observed traveling ahead of the maltose spot (Fig. 1). This anomalous sugar was not due to any synthesizing action of the enzyme preparation or experimental conditions as seen by its absence in the control. It was thought that this sugar might be the nigerose isolated by other workers (12,18). It gave a definite violet color with the aniline-diphenylamine reagent as compared to the deep blue color of the malto-oligosaccharides and the gray color given by

TABLE I
COLOR REACTIONS OF SEPARATED SUGARS

REAGENT	SPECIFICITY	ANOMALOUS DISACCHA- RIDE	MALTOSE MALTOTRIOSE	MALTULOSE ^a
Diphenylamine	Sugars	+	+	+
Aniline phthalate	Aldoses	-	+	-
Benzidine	Aldoses	-	+	-
Alpha-naphthol	Ketoses	+	-	+
Orcinol	Ketoses	+	-	+

^a Authentic sample.

nigerose (16). The color reaction of this sugar on paper was tested with various specific reagents (Table I). It is a ketose-containing sugar with the ketose group on the reducing end of the molecule, as indicated by no reaction to the aldose reagents.

Fermentation of a portion of the original concentrate with baker's

yeast removed this anomalous sugar. Nigerose is not fermented by baker's yeast (9).

On fractionation of a portion of the concentrate (20 g. solids) on a charcoal-Celite column, the first disaccharide eluted was not solely maltose. The fraction containing this anomalous disaccharide was collected separately and further fractionated by chromatography on heavy paper. The disaccharide was obtained by elution of the proper area with water, about 0.98% yield, and shown by further chromatography to be homogeneous.

It was suspected this sugar might be maltulose (4-*O*-alpha-D-glucopyranosyl-D-fructose). A sample of maltulose was prepared by isomerization of maltose in lime water (15) and compared with the unknown sugar (Tables I and II). The unknown appears to be maltulose.

TABLE II
CONSTANTS OF THE DISACCHARIDES

SUGAR	R_G^a	M_G^b	PERCENT R.P. ^c OF GLUCOSE	FRUCTOSE %	$[\alpha]_D^{25}$ WATER
Anomalous disaccharide	0.67	0.20	41.3	42.3	+52.4°
Maltulose (authentic)	0.66	0.20	40.1	44.5	+52.1°
			41.7	47.7	+52.0 ^{od}
Maltose	0.59	0.30	43.7	0	+136.0 ^{oe}

^a R_G is migration on paper referred to glucose.

^b M_G is electrophoretic mobility referred to glucose.

^c Percent r.p. is percent reducing power with alkaline ferricyanide referred to glucose.

^d See ref. 15.

^e Nigerose + 87.0° (ref. 9); turanose + 75.8° (ref. 10).

The ketose nature of the reducing moiety of this sugar was further established as it consumed 72% less iodine than maltose. No attempt at crystallization was made.

On electrophoresis of the disaccharide fraction of the digest, maltulose and maltulose were the only two sugars separated, the maltulose traveling ahead of maltulose in this case. Nigerose, which has a much higher mobility than maltulose, was not found. This does not eliminate the possibility of (1→3)-linkages in starch as they may exist in the trisaccharide or limit dextrin portions of the digest. Partial fractionation of the trisaccharide fraction has revealed the presence of a ketose-containing trisaccharide.

Maltulose has been reported in alpha-amylolytic hydrolysates of rabbit-liver glycogen, about 5% yield (10). Determination of fructose in our digests showed 1.76%. The likelihood that fructose is a minor component of starch should not be neglected, as small amounts of fructose have been found in cellulose (6) and dextran (17). Also,

ketonic groups might be introduced into the starch by oxidation at the C-2 position of a glucose residue (3). It would appear that fructose can occur in waxy corn starch.

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