Isolation and determination of maltooligosaccharides

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Maltooligosaccharides, up to maltooctaose, were converted to the corresponding 4-nitrophenylhydrazones and in this form effectively resolved by paper chromatography. A method of determination of maltooligosaccharides in mixtures was elaborated which includes elution of the separated 4-nitrophenylhydrazones from the paper and their spectrophotometric measurement. The analytical procedure was employed for assays of amylolytic activities in urine of healthy persons and patients suffering from acute pancreatitis. Isolation of [U-^{14}C]maltooligosaccharides from a [U-^{14}C]starch hydrolysate *via* 4-nitrophenylhydrazones is also described.

Мальтоолигосахариды, от мальтозы до мальтооктозы, очень хорошо разделяются бумажной хроматографией после перевода в соответствующие 4-нитрофенилгидразоны. Их определение состоит в вымывании 4-нитрофенилгидразонов сахаридов из хроматограмм и спектрофотометрическом измерении. Метод определения был применен для изучения амилолитической активности в моче здоровых людей и больных острым панкреатитидом. Далее описывается изолирование [U-^{14}C]мальтоолигосахаридов в форме их 4-нитрофенилгидразонов, полученных из гидролизатов [U-^{14}C]крахмала.

A large-scale resolution of maltooligosaccharides can be accomplished on charcoal-celite columns using suitable water—ethanol gradients [1, 2] or on columns of Biogel P-2 [3] or ion exchanger XE 200 [4] eluted with water. Qualitative proof as well as quantitative determination of maltooligosaccharides was in number of cases based upon their separation by paper chromatography [5—7]. A rapid information about composition of oligosaccharide mixtures derived from α-glucans is achieved by chromatography on thin layers of cellulose [8], celite [9], silica gel [10] or mixtures of silica gel and celite [11]. Separation and determination of maltooligosaccharides in the form of their 4-nitrophenylhydrazones is the subject of the present communication.

In order to separate and determine D-glucose and maltooligosaccharides present in mixtures, the saccharides were condensed with 4-nitrophenylhydrazine in a methanol—water solution (4 : 1, v/v) to give the corresponding 4-nitrophenyl-

hydrazones which were rapidly and effectively resolved by paper chromatography (Table 1). Brightly yellow zones of individual 4-nitrophenylhydrazones were cut out and eluted from the paper with water and the eluates used for their spectrophotometric determination. Equimolar amounts of D-glucose, maltose,

Table 1

Paper chromatography of 4-nitrophenylhydrazones of maltooligosaccharides (Whatman No. 1 paper, elution system 1-butanol—ethanol—water 5 : 1 : 4, 24—30 h, 20—24°C)

4-Nitrophenylhydrazone of	R*	R**
D-Glucose	1.00	3.43
Maltose	0.63	2.00
Maltotriose	0.43	1.50
Maltotetraose	0.30	1.00
Maltopentaose	0.18	0.62
Maltohexaose	0.11	0.39
Maltoheptaose	0.06	0.21
Maltooctaose	0.035	0.12
4-Nitrophenylhydrazine	1.60	5.49

*Related to mobility of D-glucose 4-nitrophenylhydrazone.

**Related to mobility of D-glucose.

(D-Glucose 4-nitrophenylhydrazone is a suitable standard for comparison of mobility of 4-nitrophenylhydrazones of D-glucose, maltose, and maltotriose, while D-glucose for comparison of mobility of 4-nitrophenylhydrazones of higher maltooligosaccharides.)

Table 2

Mole and weight ratios of D-glucose and maltooligosaccharides in the starch hydrolysate

Saccharide	Ratio	
	mole	weight %
D-Glucose	1.000	32.4
Maltose	0.313	19.3
Maltotriose	0.147	13.3
Maltotetraose	0.073	8.7
Maltopentaose	0.054	8.1
Maltohexaose	0.042	7.5
Maltoheptaose	0.027	5.6
Maltooctaose	0.021	5.1

maltotriose, maltotetraose, and maltopentaose after being converted to 4-nitrophenylhydrazones and resolved by paper chromatography exhibited almost the same molar absorption coefficients ($\varepsilon = 9600 \pm 200$). An example of analysis of a starch hydrolysate, the so-called confectionary sirup (used in food industry), demonstrates the possibility to determine mole, weight or percentual ratios of D-glucose and maltooligosaccharides (Table 2). Addition to analyzed sample of a known amount of D-glucose as an internal standard, enables to establish the actual content of D-glucose (Table 3) and maltooligosaccharides (Table 4). The results of determination of reducing saccharides in the tested starch hydrolysate (Table 4, *a*) and the calculated values of reducing power (Table 4, *c*) are in good consonance with the values of reducing power of the starch hydrolysate determined iodometrically according to *Schoorl* [12*a*] (35.5% counted as D-glucose).

Table 3

Determination of D-glucose in a mixture of maltooligosaccharides with the aid of different amounts of added D-glucose as an internal standard

a %	Absorbance		D-Glucose %
	E_{g2}	E_{m2}	
4.73	2.19	0.56	21.5
9.16	2.46	0.54	21.7

$E_{g1} = 1.00$; $E_{m1} = 0.31$.

Table 4

Content of D-glucose and maltooligosaccharides in the starch hydrolysate (*a*), absolutely dry starch hydrolysate (*b*) and calculated reducing power of saccharides (*c*)

Saccharide	*a*	*b*	*c*
	%		
D-Glucose	21.6	26.5	21.6
Maltose	12.9	15.8	6.7
Maltotriose	8.9	10.9	3.2
Maltotetraose	5.8	7.1	1.6
Maltopentaose	5.4	6.6	1.2
Maltohexaose	5.0	6.1	0.9
Maltoheptaose	3.7	4.5	0.6
Maltooctaose	3.4	4.2	0.5

Table 5

Products of glycogen degradation by amylolytic system in urine of healthy persons and patients suffering from acute pancreatitis

Tested sample		Content of		
		maltose	maltotriose	maltotetraose
		%		
Urine of healthy persons	*1*	15.3	47.5	37.2
	2	21.7	50.6	27.7
	3	37.0	45.8	16.8
	4	25.3	48.3	26.4
	5	33.5	48.7	17.8
Pathological urine	*1*	53.2	46.8	Traces
	2	58.3	41.7	Traces
	3	52.7	47.3	0
	4	50.3	49.7	0
	5	52.4	47.6	Traces

The analytical method can be applied for instance, for examination of purity of maltose or other maltooligosaccharides. It can also be useful in examination of amylolytic activities in urine of healthy persons and those suffering from acute pancreatitis. Urine of healthy individuals contains enzymes splitting glycogen to maltotetraose, maltotriose, and maltose. A characteristic feature of a pathological urine is hydrolytic cleavage of glycogen to maltotriose and maltose (Table 5).

The effective resolution of mixtures of lower maltooligosaccharides in the form of 4-nitrophenylhydrazones was employed for isolation of [U-^{14}C]-labelled maltooligosaccharides up to maltopentaose from enzymic hydrolysate of α-[U-^{14}C]-glucan. Under the conditions of enzymic hydrolysis, α-glucan isolated from an alga *Chlorella sp.* gave 29—34% of maltose, 19—22% of maltotriose, 10—13% of maltotetraose, and 10—12% of maltopentaose (yields are based on original radioactivity of α-glucan). From the hydrazones the corresponding maltooligosaccharides were liberated on treatment with benzaldehyde. After purification by paper chromatography they were isolated in about 50% yield (referred to radioactivity of 4-nitrophenylhydrazones).

Experimental

The starch hydrolysate used in the work was a confectionary sirup according to Czechoslovak standard No. 566160*b*/1964. Chromatographically homogeneous maltooligosaccharides used as standards, maltose, maltotriose, maltotetraose, and maltopentaose,

were prepared by fractionation of the starch hydrolysate on a charcoal-celite column [1, 2]. Glycogen from oyster was product of J. T. Baker Chemical Co., USA, and water-soluble α-[U-^{14}C]-glucan of a glycogen type isolated from an alga *Chlorella sp.* was from the Institute for Development, Production and Use of Radioisotopes, Prague. α-[U-^{14}C]-Glucan was hydrolyzed by α-amylase *ex saliva* immobilized on a cellulose carrier by the described procedure [13] modified as follows: An aqueous suspension of oxidized cellulose (1 g treated with 0.13 g of $NaIO_4$) was mixed with α-amylase (Koch-Light, 10 ml, specific activity 12.5 U/ml or 0.42 U/mg) and stirred at room temperature for 20—24 h. After adjusting the mixture to pH 8, it was stirred for another hour. The insoluble carrier with bound α-amylase was then filtered off and washed with water and a buffer (pH 5) until all soluble enzyme activity was removed.

Qualitative test for maltooligosaccharides

Starch hydrolysate (1 g) was dissolved in water (2 ml), and mixed with methanol (8 ml) and 4-nitrophenylhydrazine (0.5 g). The mixture was heated at 60°C for 7 h, then filtered and chromatographed on paper (Table 1).

Determination of D-*glucose and maltooligosaccharides*

Determination of ratios

Starch hydrolysate was reacted with 4-nitrophenylhydrazine as described above. After chromatographical resolution the brightly yellow zones of individual 4-nitrophenylhydrazones were cut out and eluted with water. The eluates were adjusted to known volumes with water and their absorbance determined spectrophotometrically at 380 nm. The absorbance values corresponding to mole ratios of the present saccharides were referred to a unit absorbance of D-glucose 4-nitrophenylhydrazone (Table 2).

Determination of D-*glucose*

Concentration of D-glucose in the starch hydrolysate was calculated from the equation

$$\%\text{D-glucose} = \frac{E_{g1} \cdot E_{m2}}{E_{g2} \cdot E_{m1} - E_{g1} \cdot E_{m2}}\, a$$

which is based on measurements of absorbances of D-glucose 4-nitrophenylhydrazone (E_{g1}), maltose 4-nitrophenylhydrazone (E_{m1}) as described for determination of mole and weight ratios in the original sample and absorbances of D-glucose 4-nitrophenylhydrazone (E_{g2}) and maltose 4-nitrophenylhydrazone (E_{m2}) in the sample to which a known amount, a, of D-glucose (expressed as %) was added. An example of such a determination of D-glucose and its reproducibility is presented in Table 3.

Determination of the content of saccharides

Taking into consideration the results of D-glucose determination (21.6%), dry weight of the analyzed starch hydrolysate (81.5% refractometrically and 81.3% by the method of K. Fischer [12*b*]) and the weight ratio of maltooligosaccharides (Table 2), true content of saccharides in the starch hydrolysate can be established (Table 4).

Examination of α-amylolytic activities in urine

Oyster glycogen (25 mg) dissolved in 0.05 M phosphate buffer (pH 7, 1 ml) was incubated with a sample of tested urine (50 μl) at room temperature for 4 days. After addition of methanol (4 ml) and 4-nitrophenylhydrazine (20—30 mg), the mixture was heated at 60°C for 5—6 h and then chromatographed on one leaf of Whatman No. 1 paper in *n*-butanol—ethanol—water (room temperature, 20 h). After chromatographic separation the ratio of maltooligosaccharides was determined spectrophotometrically (Table 5).

Isolation of [U-^{14}C]maltooligosaccharides from hydrolysate of α-[U-^{14}C]-glucan

A solution of glycogen (50 mg) and water-soluble α-[U-^{14}C]-glucan (10 μCi as a radioactive tracer) in water (2 ml) was incubated with α-amylase immobilized on a cellulose carrier (5 mg, 4—8 U/mg) at room temperature for 3—5 days. The immobilized enzyme was then filtered off and the filtrate mixed with 2% methanolic solution of 4-nitrophenylhydrazine (4 ml) and heated at 60°C for 7 h. The mixture was chromatographed, the yellow zones of 4-nitrophenylhydrazones were cut out of the paper and eluted with water (10 ml) for 30 min at room temperature. After addition of methanol (1 ml) and benzaldehyde (1 ml), each fraction was heated at 60°C for 15 h and filtered. The filtrates were concentrated under reduced pressure (at 40°C) and chromatographed in 1-butanol—ethanol—water at room temperature for 3—6 days. Radioactive zones of the paper were eluted to give pure maltooligosaccharides in about 50% yields.

Radioactivity was measured on a scintillation spectrometer Packard, type 3330 (USA) using a toluene scintillation liquid (Tesla, Přemyšlení, Czechoslovakia).

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