Journal of Chromatography, 150 (1978) 242–245

CHROM. 10,436

Note

Fractionation of maltosaccharides of relatively high degree of polymerization by multiple descending paper chromatography

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(Received July 19th, 1977)

We have previously reported several improved techniques for fractionation and determination of maltosaccharides using improved carbon column chromatography[1], paper densitometry[2], gas–liquid chromatography[3] and gel filtration chromatography[4]. These studies were closely related to our work on the fine structure of the starch molecule[5,6] and on the action pattern of amylases[7].

Although we employed the Bio-Gel P-2 gel filtration technique[4] to fractionate the oligosaccharides up to a degree of polymerization (DP) 12–14, we often observed that the fractions obtained by gel filtration were not homogeneous when checked by paper chromatography, especially for a DP higher than 15.

We have attempted to use descending paper chromatography to determine the chain length distribution of Nägeli amylodextrin[8], gel filtration of which gave only poor fractionation (three peaks)[9]. Using multiple descending paper chromatography, we achieved quantitative fractionation up to DP 25.

The advantages of multiple over single descending paper chromatography are (i) each spot is dense, round and not diffused, giving the better resolution essential for quantitative determination; and (ii) the solvent system can be changed at each descent, resulting in better resolution of the longer oligosaccharides.

This paper summarizes our results on the fractionation of maltosaccharides using multiple descending techniques.

EXPERIMENTAL

Preparation of maltosaccharides

Short-chain amylose ($\overline{DP}$ 23), a gift from Hayashibara Biochemical Laboratory (Okayama, Japan), was partially hydrolysed by *Bacillus subtilis* saccharifying α-amylase. The reaction was stopped by boiling, then an equal volume of ethanol was

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added to precipitate out unchanged higher molecular weight residue. The supernatant was evaporated *in vacuo* at 50° to 5% of carbohydrate concentration. Samples of 20–30 μl were applied to Toyo filter paper No. 50, and the paper was irrigated in the descending mode. The chromatogram was treated by glucoamylase–acetone aqueous solution[10] to enhance the spot intensity of the higher oligosaccharides, and then by the silver nitrate dip method[11].

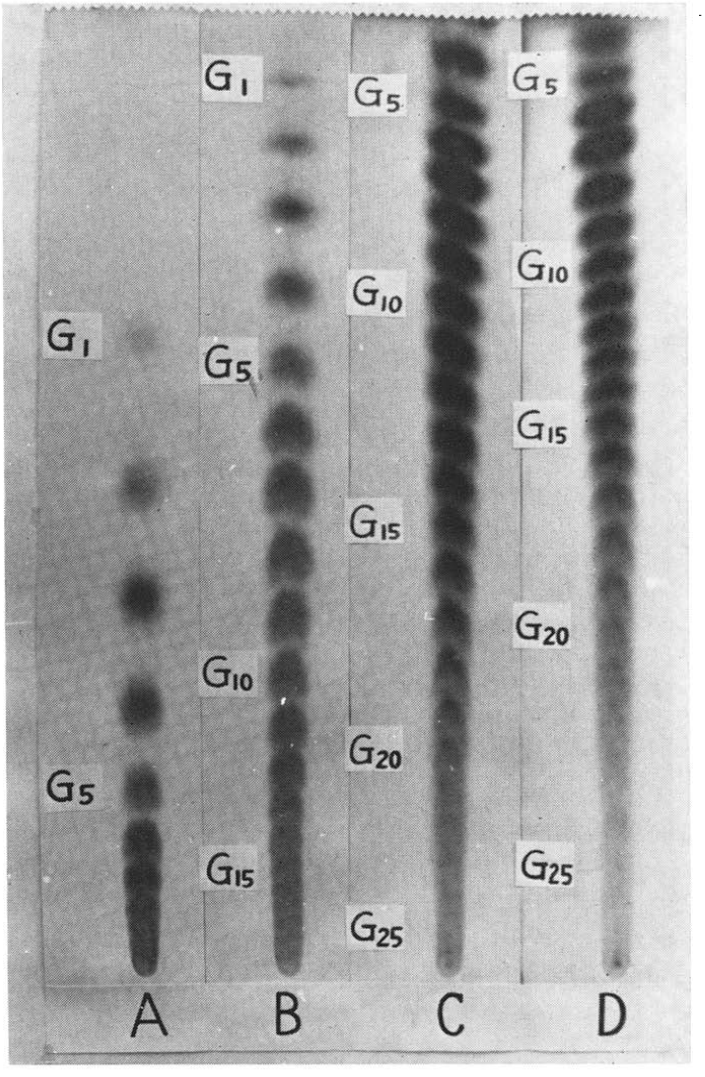

Fig. 1. Fractionation of maltosaccharides of higher degree of polymerization by multiple descending method. G_1, G_5, etc., are glucose, maltopentaose, etc. A: 40 h irrigation with solvent I (1-butanol–pyridine–water, 6:4:3 v/v). B: Three descents using solvent II (1-butanol–pyridine–water, 1:1:1) after A, 20 h for each. C: Four descents using solvent III (1-butonol–pyridine–water, 1:1:1.45) after B, 20 h for each. D: Two descents using solvent IV (1-butanol–pyridine–water, 1:1:1.9) after C, 20 h for each.

Irrigation solvents

The paper was irrigated first by solvent I (1-butanol–pyridine–water, 6:4:3, v/v/v), for 40 h at 16°, when the impurities absorbed in the filter paper were washed out and the oligosaccharides up to G_6 were clearly separated (Fig. 1A). After being dried completely, the chromatogram was irrigated three times, 20 h for each run, by solvent II (1-butanol–pyridine–water, 1:1:1), resulting in separation of oligosaccharides up to G_{14} (Fig. 1B). Solvent III (1-butanol–pyridine–water, 1:1:1.45) was used for four descents of 20 h each to separate up to G_{25} (Fig. 1C). By increasing the water content of the solvent, we were able to separate oligosaccharides of higher DP. Solvent IV (1-butanol–pyridine–water, 1:1:1.9) was used for two descents to improve the separation of G_{20}–G_{25} (Fig. 1D).

RESULTS

Resolution of oligosaccharides

The operating temperature affected the speed of the solvent front and sugar spots, but not the resolution of the oligosaccharides. It took 25, 20, and 15 h at 5, 15 and 25°, respectively, for the solvent front to reach the end of the filter paper (52 cm from the point of sample application).

Recovery of saccharides

In order to determine the recovery of maltosaccharides from the chromatogram, the maltosaccharide mixture described above was spotted on the filter paper (Toyo No. 50) and irrigated twice by the solvent I. Each spot was cut, then extracted in distilled water in a test tube in boiling bath for 15 min. The carbohydrate content of the each fraction was determined by the phenol–sulphuric acid method[12]. The amount of carbohydrate applied to the paper was 661 μg, and the total recovered was 693 μg, which was equivalent to 105% recovery (see Table I). Over several experiments, we obtained the range of recovery 96–105%.

TABLE I

RECOVERY OF MALTO-OLIGOSACCHARIDES FROM PAPER CHROMATOGRAM BY EXTRACTION WITH BOILING WATER

Total carbohydrate applied to the paper was 661 μg. Each spot was cut, then extracted with boiling water for 15 min. The phenol–sulphuric acid method of carbohydrate determination was used.

Carbohydrate	*Amount recovered (μg)*
G_1	6
G_2	28
G_3	76
G_4	48
G_5	28
G_6	84
G_7	98
G_8	66
G_9	50
G_{10}	35
$\geqslant G_{11}$	174
Total	693 (105%)

This method is thus very useful, although time-consuming, for the quantitative fractionation of oligosaccharides up to DP 15–20.

Branched saccharides (DP n) moved slightly slower than linear oligosaccharides (DP n) of the same molecular weight, and appeared between linear oligosaccharides DP n and $n + 1$.

In our experience[13], high-performance liquid chromatography successfully fractionates maltosaccharides only up to DP 7 or 8.

ACKNOWLEDGEMENT

The authors are grateful to Dr. Shigeo Suzuki, National Food Research Institute, for his encouragement and discussion.

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