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Note

Thin-layer chromatographic method for identification of oligosaccharides in starch hydrolyzates

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The identification of breakdown products resulting from the enzymic hydrolysis of starch requires a fast and positive method such as thin-layer or paper chromatography. However, paper chromatography is often time-consuming and the number of samples that can be applied to each chromatogram is limited; comparing the individual spots with standards can also be difficult.

In recent years, TLC has gained considerable ground because it is rapid and because a large number of samples and standards can be applied to each plate. Many methods for the separation of mono- and oligosaccharides have been described[1–7].

The TLC method described here is a new analytical procedure for the identification of di-, tri- and oligosaccharides in starch hydrolyzates; it is a development of existing methods, but has been improved by the inclusion of lactic acid in the solvent system. Lactic acid has the surprising effect that nearly all monosaccharides run ahead of it in the TLC system; this produces considerably better separations of oligosaccharides than are attainable with previously described methods.

PROCEDURE

To ensure the greatest reproducibility, pre-coated silica gel 60 plates (Merck Art. 5715; 20 × 20 cm) are used. The plates are conditioned in an oven at 105° for 1 h and allowed to cool in a desiccator before use; 1-μl samples are then applied to the plate. A suitable concentration for mono- and oligosaccharide standard solutions is 0.1%.

When the sample spots have dried, the plates are placed upright in a developing tank; the solvent system is propan-2-ol–acetone–1 *M* lactic acid (4:4:2), which should be freshly prepared 1 h before use. After 3 h, the solvent front will have migrated approx. 13 cm; the plate is then removed from the tank and dried in a stream of warm (60°) air. To locate the sugar spots, the plate is sprayed with a reagent consisting of aniline (4 ml), diphenylamine (4 g), acetone (200 ml) and 85% H_3PO_4 (30 ml), then set aside in an oven at 105° for 1 h. The individual sugars appear as blue spots on a white background.

DISCUSSION

This method has been used for the separation of mono- and oligosaccharides

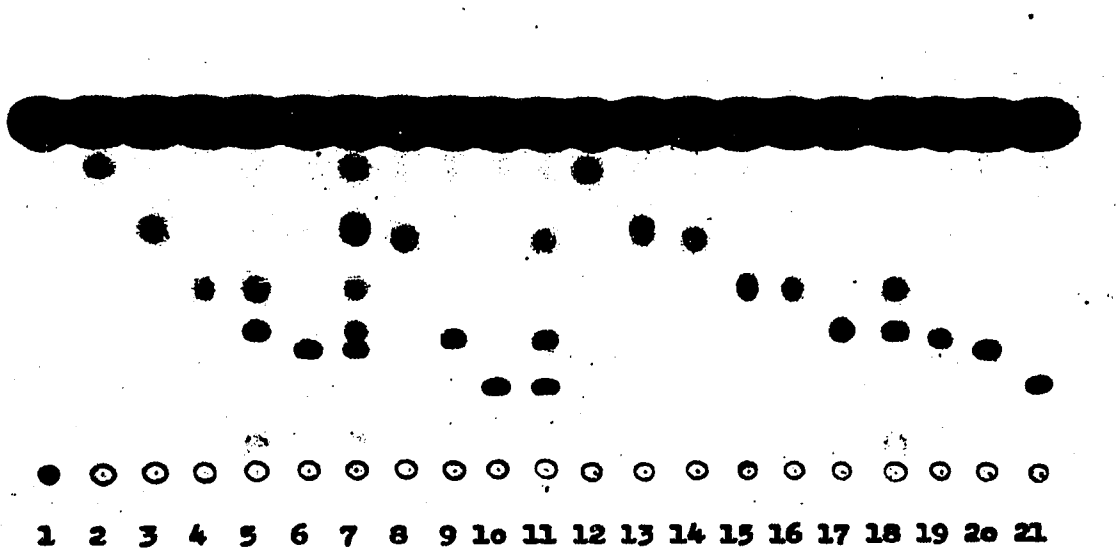

Fig. 1. Chromatogram of mono- and oligosaccharides. Samples 1–21 contained 5% of glucose, and, in addition 0.1% of the following sugars: 2, maltose; 3, maltotriose; 4, maltotetraose; 5, maltotetraose + maltopentaose; 6, maltohexaose; 7, mixture of 2–6; 8, isomaltose; 9, isomaltotriose; 10, isomaltotetraose; 11, mixture of 8–10; 12, maltose; 13, maltotriose; 14, isomaltose; 15, panose; 16, maltotetraose; 17, 6^3-α-glucosylmaltotriose; 18, maltotetraose + maltopentaose; 19, isomaltotriose; 20, maltohexaose; 21, isomaltotetraose.

TABLE I

R_F VALUES OF MONO- AND OLIGOSACCHARIDES ON SILICA GEL 60

Sugar	R_F *value*	*Sugar*	R_F *value*
2-Deoxyribose	0.86	Cellobiose	0.56
Rhamnose	0.85	Melezitose	0.56
N-Acetylglucosamine	0.79	Lactose	0.47
Xylose	0.76	Maltotriose	0.46
Fucose	0.75	Isomaltose	0.44
Ribose	0.72	Raffinose	0.42
Sorbose	0.69	Melebiose	0.36
Mannose	0.69	Panose	0.35
Arabinose	0.67	Maltotetraose	0.34
Sucrose	0.66	Glucosamine hydrochloride	0.34
Glucose	0.65	6^3-α-Glucosylmaltotriose	0.28
Fructose	0.64	Maltopentaose	0.27
Galactose	0.58	Isomaltotriose	0.25
Trehalose	0.57	Maltohexaose	0.23
Maltose	0.57	Isomaltotetraose	0.17

on a routine basis for the identification of breakdown products from the enzymic hydrolysis of starch. The method is more suitable for identifying oligosaccharides than were previous methods because monosaccharides (except for galactose) and saccharose run either ahead of or with the lactic acid front (R_F 0.65) and the oligosaccharides therefore run further than previously described. Moreover, the oligosaccharides run as more concentrated spots, with sharp divisions between the individual sugars. This can be seen from the chromatogram reproduced in Fig. 1; some R_F values obtained in the system used are shown in Table I.

As little as 0.05 μg of glucose in a spot can be resolved, but smaller amounts give spots that are too diffuse for positive identification. If there appear to be two sugars running together, it is often advantageous to dilute the sample 5–10 times and run a fresh chromatogram; this usually gives satisfactory results.

NOTE ADDED IN PROOF

In 1974 E. Merck, Darmstadt, G.F.R., altered their process for preparing pre-coated silica gel 60 plates. Merck Art. 5715 no longer gives such well defined spots as illustrated in Fig. 1. Satisfactory results have been obtained using TLC aluminium sheets silica gel 60 Merck Art. 5553; 20 × 20 cm.

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