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Note

A simple method for the detection and determination of trehalose by spot elution paper chromatography

GEORGE CALDES and BENJAMIN PRESCOTT

Laboratory of Microbial Immunity, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Md. 20014 (U.S.A.)

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Numerous studies on a wide range of natural products as potential antimicrobial and antitumor agents have been carried out in our laboratory. While studying the use of aniline salts in the determination of carbohydrates in extracts of these materials by spot elution paper chromatography, it was found that trehalose, a non-reducing disaccharide consisting of two glucose residues, did not produce any color when sprayed with solutions of aniline salts commonly used for the determination of carbohydrates by paper chromatography, even on prolonged heating of the chromatograms[1]. Since trehalose is very widely present in nature[2], a convenient method of estimating this carbohydrate on paper chromatograms was thus desirable. Sucrose has been hydrolyzed on paper chromatograms by spraying the paper with invertase solution[3]. A very active trehalase is known to be present in honeybees[4]. Spraying the carbohydrate chromatograms with an aqueous extract of honeybees (*Apis mellifera*) before treatment with aniline salt yielded sufficient color so that trehalose could be identified and estimated along with the other carbohydrates in a mixture. To the authors' knowledge, trehalose has never before been estimated by spot elution chromatography.

METHODS AND RESULTS

50 g of frozen honeybees were ground in a Waring blender with 100 ml of cold water for 5 min. The homogenate was centrifuged at 7000 rpm for 30 min, and the cloudy dark amber supernate lyophilized. The lyophilized residue was taken up in 15 ml of 1 *M* phosphate buffer pH 7.0 and applied to a DEAE-Sephadex G-25 column (45 × 2 cm), and eluted with 300 ml of the same buffer. This eluent was used to spray the paper chromatograms. The column procedure was necessary to purify the crude extract; otherwise the background color of the chromatograms turned dark brown when heated with the 2% ethanolic aniline malonate salt solution used to detect and estimate the carbohydrates. A large amount of black pigmented material remained behind on the column after the elution.

The papers were sprayed evenly with enough enzyme solution to moisten the chromatograms thoroughly. The sprayed chromatograms were sandwiched between sheets of plastic film to keep them from drying, and incubated at 37° for 3 h. If the

papers were allowed to dry, the enzyme treatment was not effective. After 3 h, the chromatograms were dried at room temperature, dipped in 2% aniline malonate, allowed to hang in a hood for 45 min, and heated at 100° for 45 min. This procedure was sufficient to bring out enough color so that trehalose could be determined as well as the other carbohydrates. The enzyme application darkened the background color only slightly. Fig. 1 compares two halves of the same chromatogram, one half of which was enzyme-treated.

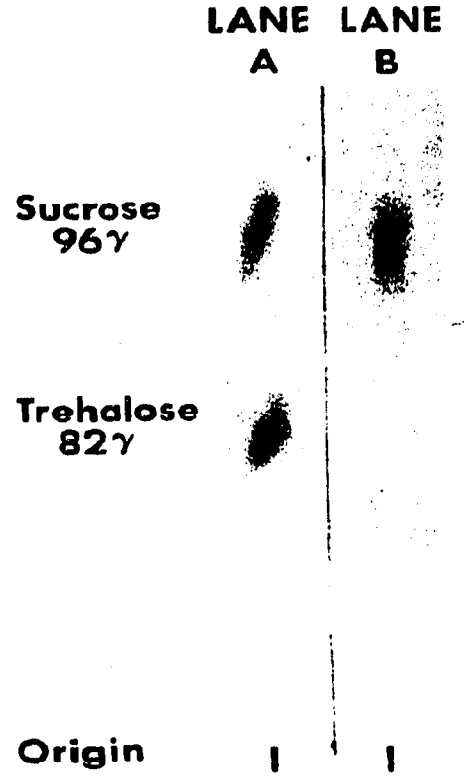

Fig. 1. Paper chromatogram of a mixture of sucrose and trehalose. Chromatography for 24 h on Whatman No. 4 paper with solvent system ethyl acetate–pyridine–water (180:50:57.5). Lane A of the paper was treated with enzyme before treatment with aniline malonate. Lane B was treated directly with malonate.

Trehalose was readily separated from the common monosaccharides, and from sucrose, maltose, and raffinose by ethyl acetate–pyridine–water (180:50:57.5), on Whatman No. 4 paper. Whenever trehalose is present on a paper chromatogram, it can be distinguished from the other common carbohydrates by the fact that it must be pre-treated with enzyme to yield color with aniline salts. Although sucrose will yield color with aniline malonate upon prolonged heating, the color of the sucrose spots appears more quickly on the enzyme-treated papers, indicating the presence of an invertase as well as a trehalase in the bee extract.

The above method was used to estimate the free trehalose content of lyophilized residues of aqueous extracts of dried baker's and brewer's yeasts (both obtained from Sigma, St. Louis, Mo., U.S.A.) and fresh, edible mushrooms. The extracts had been prepared by grinding the raw material with water in a Waring blender and removing the insolubles by centrifugation and filtration. Only the baker's yeast extract was found to contain free trehalose (Fig. 2), 2% of the weight of the original dried yeast. This was the only free carbohydrate found, except for a trace of glucose in the mushrooms.

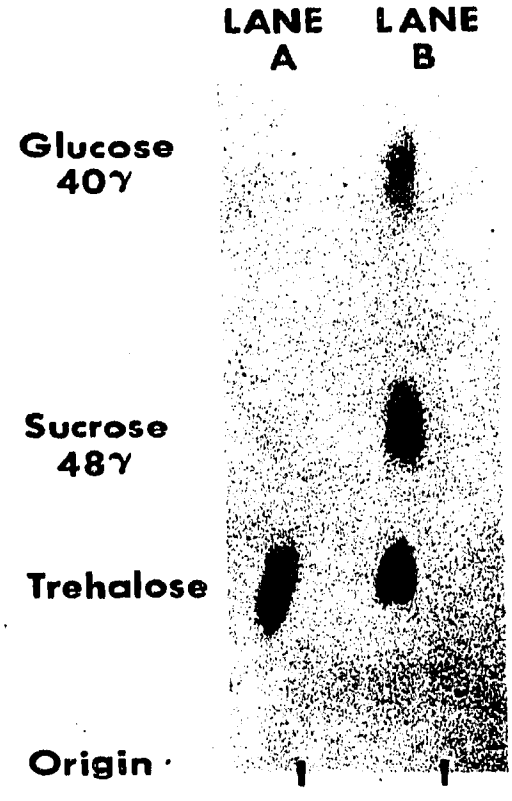

Fig. 2. Paper chromatogram of an aqueous extract of baker's yeast 2500/ig (Lane A) and carbohydrate standards, glucose 40/ig, sucrose 48/ig, and trehalose 72/ig (Lane B). Both lanes were treated with bee extract (on Whatman No. 4 paper). The solvent system used was the same as that in Fig. 1. Time, 18 h.

Extracts of baker's yeast and brewer's yeast prepared similarly to the bee enzyme extract showed no trehalase activity on paper chromatograms.

Extracts of cicadas were also tested as a source of enzyme since these large insects were easily available, but only a very slight trehalase activity was detected.

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