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Rapid staining of proteins and peptides in starch gels by chlorination

The detection of proteins and peptides by chlorination has become a standard procedure in paper electrophoresis and chromatography. Direct extension of this method to gel media presents difficulties because of the time required to wash out the excess of chlorinating agent. Recently, however, BOREL *et al.*[1] have applied the reaction in a peptide analyser by destroying excess hypochlorite with hydrazinium sulphate. As will be shown below, this modification is applicable to starch-gel electropherograms in the absence of urea and other interfering substances.

Materials

Sodium hypochlorite solution was a commercial product (Lab. Supply, Melbourne). Other chemicals were laboratory reagent grade except hydrazinium sulphate, which was of analytical reagent grade (BDH). Hydrolysed starch was obtained from Connaught Laboratories, Toronto.

Procedure

After electrophoresis the starch gel is cut to give slices 1.5 mm thick. Hypochlorite solution is diluted to approximately 0.1 *M* with 0.2 *M* sodium phosphate buffer, the pH being adjusted to about 7. The gel slice is agitated in this solution for 1 min, the solution is replaced with an aqueous 1% solution of hydrazinium sulphate and agitation is continued until a corner cut from the gel no longer stains in potassium iodide solution (about 2–3 min at 20°). The hydrazinium sulphate is replaced with aqueous 10% potassium iodide, which finally is replaced with deionized water. The stain is stable for several days.

If the formation of bubbles of nitrogen in the gel is to be avoided, the gel slice can be soaked for 1 min in 1% sodium dithionate after chlorination. Alternatively, an almost bubble-free gel can be obtained if the hydrazinium sulphate solution is cooled to 0°.

Results and discussion

With this staining procedure, the result of a separation by starch-gel electrophoresis can be observed about 5 min after the gel is sliced. A clear background is obtained except for the presence of the bubbles referred to above, and of small blue-black spots due possibly to impurities in the starch.

Quantitative aspects of the reaction were not studied, but it seems to be somewhat less sensitive to protein detection than staining with Nigrosine dye. However, the reaction conditions used may not be the optimum ones. Thus proteins stain more intensely if the excess hypochlorite is removed by repeated washing in 1% trichloroacetic acid instead of with hydrazinium sulphate.

This method of detection is relatively non-specific, so that buffers must be carefully selected. Care must also be taken to avoid contamination in the preparation and handling of the gels, since otherwise a heavy background stain may result. A partial list of interfering substances is given by BOREL *et al.*[1]. On the other hand, the method should be applicable to most proteins with roughly the same sensitivity. Several small

peptides, including glycylglycine, could also be detected after starch-gel electrophoresis in 1 *M* acetic acid at 40 V/cm, although the zones were up to 5 mm wide after ½ h.

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Two dimensional paper chromatography for the detection of oxidation of maltose by glucose oxidase

The present report describes a novel paper chromatographic procedure for studying the action of glucose oxidase on glucosyl oligosaccharides. With this technique, it has been shown conclusively that maltose is oxidized to maltobionic acid by glucose oxidase. Other methods for following glucose oxidase action are based on oxygen uptake[1,2] or hydrogen peroxide production[3,4] and are not suitable for use with oligosaccharides since the primary carbohydrate products cannot be identified. Further, since glucose oxidase preparations may contain contaminating glucosidase and transferase activities[4], conversion of oligosaccharides to glucose followed by oxidation of glucose may occur and may be interpreted erroneously as indicative of oligosaccharide oxidation[5]. By separating the products of glucose oxidase action on maltose on paper chromatograms, then utilizing a specific glucosidase for hydrolyzing the products directly on the chromatogram, and finally separating the products of the glucosidase reaction by chromatography in a second direction, it has been shown that maltobionic acid is the carbohydrate product of glucose oxidase action on maltose.

Materials and methods

The maltose used in all experiments was prepared from amylose via β-amylase and was free of glucose[6]. Reference maltobionic acid was synthesized by oxidation of maltose with bromine[7]. The maltobionic acid thus produced was purified by a paper chromatographic procedure using the *n*-butanol–pyridine–water solvent system to remove unoxidized maltose. Glucose and gluconic acid were obtained from commercial sources. Glucose oxidase (EC 1.1.3.4), obtained from Miles Laboratories, was purified according to the procedure described by PAZUR AND KLEPPE[8]. This glucose oxidase preparation did not contain any contaminating α-glucosidase activity. Glucoamylase (α-1,4-glucan glucohydrolase, EC 3.2.1.3) was prepared according to the methods described by PAZUR AND ANDO[9].