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USE OF CROSS-LINKED HYDROLYSED STARCH IN GEL ELECTROPHORESIS

O. MARKOVIČ and Ľ. KUNIAK
Institute of Chemistry, Slovak Academy of Sciences, 809 33 Bratislava (Czechoslovakia)

SUMMARY

A gel prepared from cross-linked hydrolysed starch preserves the optimal electrophoretical separation properties and exhibits better mechanical features than those of hydrolysed starch. Its improved mechanical strength and elasticity make it more suitable for slicing and for multiple simultaneous detections of the same sample.

INTRODUCTION

Starch gel electrophoresis was first described by Smithies[1] and is still very often used in preference to acrylamide gels for the separation of particular mixtures of proteins, as well as for its simplicity and non-toxic character[2]. It has been found particularly useful for the study of the multiple molecular forms of enzymes and isoenzymes (zymograms)[3,4].

Starch gel can be easily divided by slicing into two thin sheets[5,6], which enables the simultaneous analysis of the same sample for proteins and for enzyme activity[7]. However, the handling required by detection (staining and destaining) often causes damage to the thin slices of the gel.

The present paper describes the application of a cross-linked hydrolysed starch (CLH starch) for gel electrophoresis which exhibits better mechanical properties and preserves the optimal separation properties of hydrolysed starch.

EXPERIMENTAL

Cross-linking of the hydrolysed starch was carried out by epichlorhydrin[8], 100 g of potato starch of extra fine quality (produced by Starch Producing Factory, Brno-Dornych, Czechoslovakia) being hydrolysed by the method of Smithies[1] and cross-linked in 150 ml 0.25% NaOH solution containing 0.25 g epichlorhydrin at room temperature over 90 min. After acidifying with acetic acid, the product was washed several times with distilled water and then with acetone, and finally dried in air at room temperature.

Electrophoresis was carried out in a horizontal device constructed by the Research Workshop of the Slovak Academy of Sciences (Bratislava), which enabled

horizontal cutting of the gel. The gel was made by adding 20 g CLH starch per 200 ml 0.03 *M* Tris–HCl buffer, pH 7.5, and electrophoresis was carried out at 4° for 18 h at 3 V/cm gradient of constant voltage. A 0.3 *M* Tris–HCl buffer was used in the electrode vessels. After electrophoresis and horizontal slicing, one sheet of the gel was used for pectinesterase activity detection by means of a print with Whatman No. 1 paper impregnated with substrate (1 % pectin in 0.1 *M* NaCl adjusted to pH 7.5)

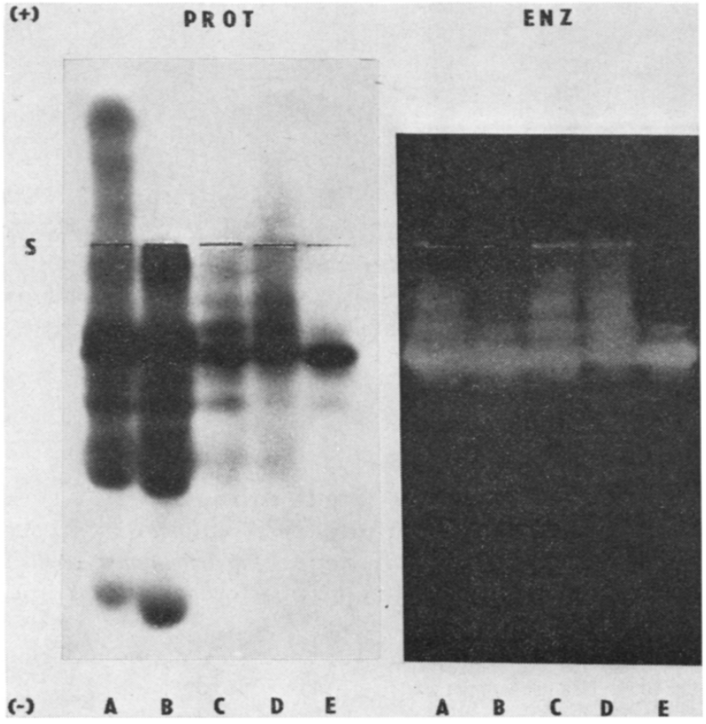

Fig. 1. Electrophoresis on CLH starch gel of tomato pectinesterase fractions during purification. PROT = Protein staining (with nigrosin); ENZ = pectinesterase activity detection (print with pectin and bromthymol blue); S = start; A = crude tomato pectinesterase after extraction, ammonium sulphate precipitation and dialysis; B = active fraction from a DEAE-Sephadex A-50 column; C and D = active fractions from a Sephadex G-75 column; E = purified fraction from a CM-Sephadex C-50 column.

and an alkaline 0.1 % solution of bromthymol blue[9]. After covering the gel with this paper, yellow spots appeared on the blue background to indicate pectinesterase in 1 min. After taking off the print, the same sheet of gel was used for protein staining (Fig. 1).

The second sheet of gel could be used for further detection (*e.g.*, lipid staining).

RESULTS

After slicing the starch gel the detection of the enzyme activity or protein staining is much clearer in the central layer of the gel than is the case without slicing.

Possible irregularities existing at the top and bottom can be avoided and the time required for staining and destaining minimized. On the other hand, handling thin slices of the gel requires some routine and care and damage or destruction of the gel often occurs. This disadvantage was diminished or removed by cross-linking the hydrolysed starch. The gel prepared from CLH starch exhibits the optimal separation properties of hydrolysed starch (comparing products of Connaught Med. Res. Lab., Canada and Serva, Heidelberg, G.F.R.) and has improved mechanical strength and elasticity.

The method was successfully used for determining the multiple forms of tomato pectinesterase[10].

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