A method for the detection of proteolytic enzymes in electropherograms

As has been described earlier[1], it is possible to localize amylase on agar electropherograms exactly and with great sensitivity with the help of a "sandwich method". This method is generally applicable to enzymes, the substrates of which can be differentiated from their degradation products by precipitation with some chemical agent. Therefore, the method can also be applied to the localization of protein-degrading enzymes, by precipitating the undegraded substrate protein with trichloroacetic acid.

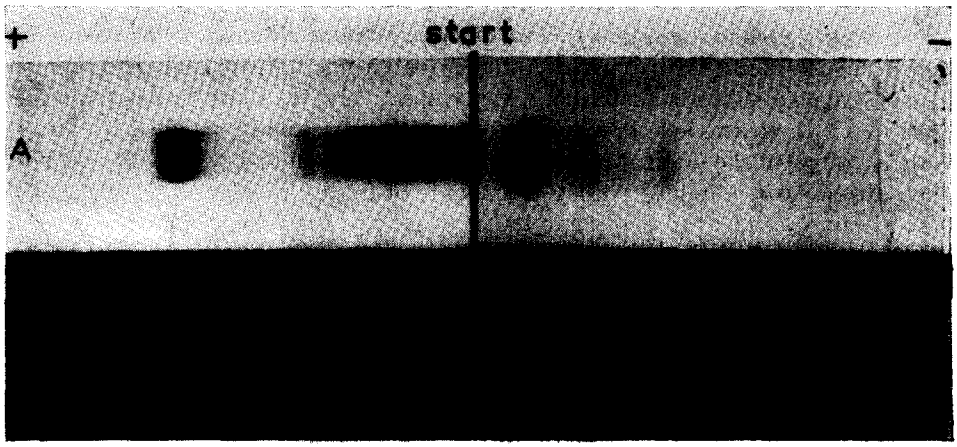

Fig. 1. Electrophoresis was carried out on microscopic slides, covered with 2 ml 1% agarose in veronal buffer pH 8.6 (μ = 0.033). Anionic side: 15 min 20 mA (approx. 20 V/cm). Cationic side: 25 min 20 mA (approx. 20 V/cm). A, The electropherogram, stained with Amido Black B. B, The cover slide after incubation and washing.

In a simpler variant of the method, we made use of exposed and developed photographic emulsions as the substrate in the upper half of the sandwich. At the sites of enzymic activity the silver grains are disengaged along with the degradation of the embedding gelatine. A similar technique has been used earlier by Adams[2] for the histochemical localization of proteases.

The photographic plates (in our case Gevaert Process plates) are exposed to daylight for a few minutes and developed in the usual manner. (Fixation was omitted; the developer was metol–hydrochinon with the addition of potassium carbonate). After repeated washings with distilled water and drying, the plates are ready for use.

The protein extract, containing the proteolytic activity, is subjected to electrophoresis on agarose on a microscope slide[3]. Directly after the electrophoresis, the surface of the agar electropherogram is blotted with filter-paper, and a photographic plate, treated as just described and cut to the same size as the electrophoresis slide, is placed, with the gelatine layer down, on to the electropherogram.

This "sandwich" is incubated for about 10 min at 37° (at 100% humidity). After the incubation the two slides can be separated very easily in water. Where the gelatine has been digested,—*viz.*, exactly opposite to the bands of proteolytic activity

in the electropherogram—the silver grains can be removed by washing with tap water. Consequently, the sites of proteolytic activity are clearly visible as transparent spots.

With this method we tested the proteolytic activity of an auto-activated rat pancreas 0.14 *M* NaCl extract (Fig. 1). In both the cathodic and the anodic parts of the electropherogram several clear bands of proteolytic activity can be distinguished.

The method is very rapid, allows a sharp localization of the enzymic activity and should also be useful with other supporting media, *e.g.*, after starch and polyacrylamide electrophoresis.

Laboratory of Histology and Microscopical Anatomy, State University Utrecht (The Netherlands)

W. J. W. VAN VENROOIJ

1 C. POORT AND W. J. W. VAN VENROOIJ, *Nature*, 204 (1964) 684.
2 C. W. M. ADAMS AND N. A. TUGAN, *J. Histochem. Cytochem.*, 9 (1961) 469.
3 R. J. WIEME, *Clin. Chim. Acta*, 4 (1959) 317.

Received December 8th, 1964

Clin. Chim. Acta, 11 (1965) 482–483

BRIEF TECHNICAL NOTE

The dyeing and clearing of cellulose acetate electropherograms

The use of dyes in alcoholic solution for staining proteins after electrophoresis on cellulose acetate requires an alcoholic wash solution to remove excess dye from the strip. The use of aqueous dye solutions followed by an aqueous washing solution has therefore the advantage of economy. For scanning, however, the finished strip needs to be made transparent and this is commonly done by impregnating the strip with an oil having a refractive index close to that of cellulose acetate.

The following method is proposed, using a dye in alcoholic solution, which is considered to be economical. The cellulose acetate strip (Sepraphore III, Gelman Instrument Co.) is placed, after electrophoresis, in a solution of 0.1% w/v Eriochrome Black T[1] in alcoholic glacial acetic acid (9 vol. + 1 vol.). The stained strip is then washed in a 15% v/v glacial acetic acid in alcohol mixture, a clearing solution for Sepraphore III, until the excess dye is removed[2]. The strip is then spread on a perspex plate, taking care to exclude all air bubbles from under the strip, and allowed to dry. The strip becomes transparent and peels away from the perspex. The strip then has the appearance of cellophane and is ready for scanning or may be fixed to a white card for reporting.

The wash solution is reclaimed by adding activated charcoal, to remove the dye washed from the strip, and filtering. Some acetic acid is adsorbed on the activated