CHROM. 5107

Activity stain for urocanase and histidase on polyacrylamide gel

Recently methods were given for the location of histidase (L-histidine ammonia-lyase, EC 4.3.1.3) and urocanase on polyacrylamide gel. To detect histidase, KLEE[1] absorbed histidine on paper which he placed on the gel. This paper was removed and analyzed for urocanate. Histidase and urocanase have also been detected by treating the gel with the substrate, and photographing the gel under an ultraviolet (UV) light[2]. Another technique for the identification of urocanase was based on the reaction of imidazolepropionate, the product of the urocanase reaction, with 2,6-dichlorophenolindophenol. This method reveals a transparent band on a blue background[2]. GORDON[3] summarizes several methods for observation of enzyme activity on gels, but he does not list any activity stain which utilizes the UV quenching ability of substrates or products.

This report presents a method for location of histidase and urocanase directly on the gel. Urocanate, the substrate or product of these enzymes, absorbs UV light and appears as a dark area on an eosin-treated gel in contrast to the fluorescent yellow area found in the absence of urocanate. A similar procedure might be adapted to detect other enzymes with a substrate or product which absorbs UV light. A preliminary report of this technique has been given[4].

Materials and methods

Pseudomonas putida A.3.12 was grown as previously described[5]. Cells were frozen in 1 g pellets, disrupted in a Hughes press and suspended in two times their volume in 0.01 *M* phosphate buffer, pH 7.0. The crude extract was obtained by centrifugation at 4°.

Crude extracts (3 mg of protein) were subjected to electrophoresis on polyacrylamide gel slabs by the method previously described[6]. The gels were stained when required with 1% Aniline Blue Black in 7% acetic acid to detect about 12 to 16 protein bands (Fig. 1B). In this method, the extract can be placed in a band at the origin, and after electrophoresis various portions of the gel slab can be stained for protein or enzyme activity, or left unstained and portions corresponding to bands excised.

Experimental

The position of histidase and urocanase in the polyacrylamide gel was located with an activity stain. To locate urocanase, a portion of the gel slab (10 × 55 mm) representing a single channel (150 μg of protein, crude extract) was excised from the larger slab and 1 ml of 0.04 *M* urocanate was applied on the surface of the gel. After 10 min at room temperature, the excess urocanate was washed off with distilled water. The gel was then flooded with 15 ml of 0.1% eosin and decanted after 5 min. When viewed under UV light, the gel had a subdued, darkened color because the fluorescence of eosin was quenched by the absorbance of urocanate (maximum 277 nm). At the position of urocanase a yellow band appeared because of the fluorescence of eosin easily visible in the absence of urocanate. The exact location of the band was observed in a Chromatovue cabinet with the 254 nm light source. By the time the

band became intense enough to photograph (Fig. 1C) it had become more diffuse.

Two histidase bands (fast, F; slow, S) were revealed when 1 ml of 0.04 *M* L-histidine was used in place of urocanate and eosin was applied before instead of after the substrate. At the locations of histidase activity dark bands appeared against a bright yellow background due to the UV absorption of the product, urocan-

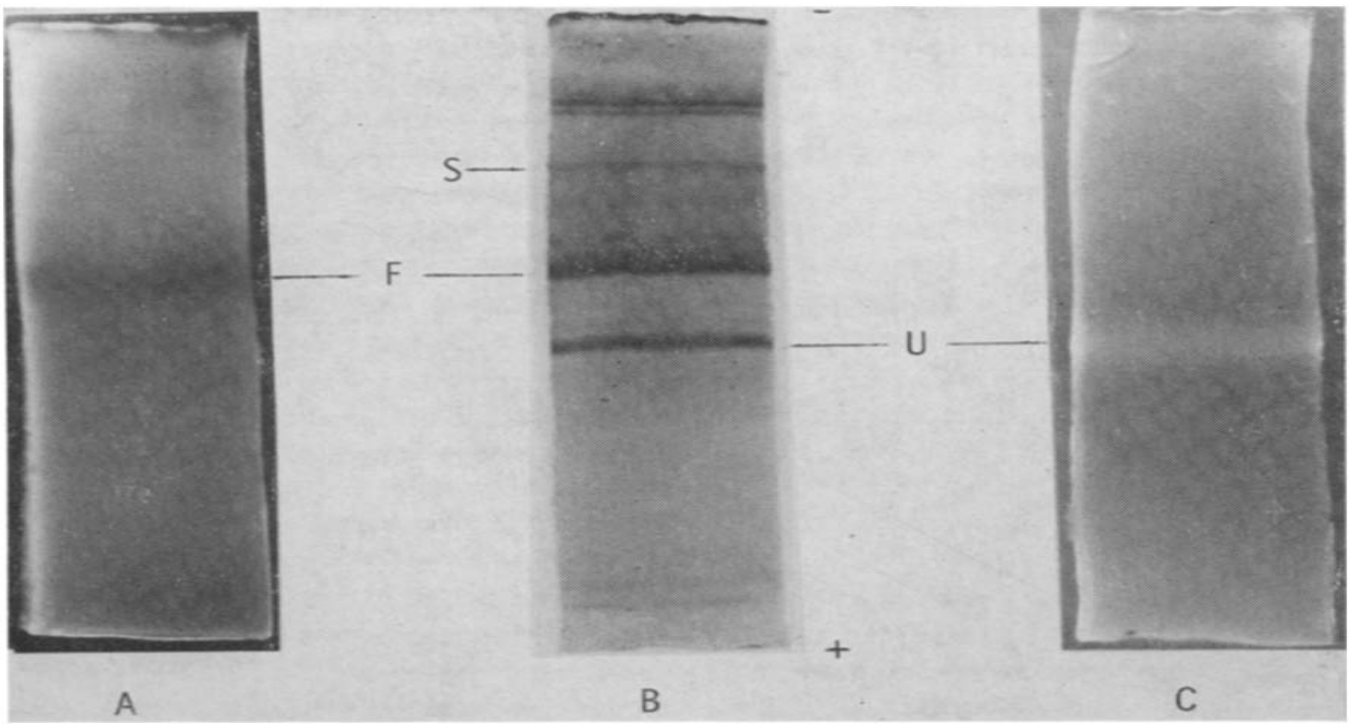

Fig. 1. Detection of histidase and urocanase bands after gel electrophoresis. (A) This photograph under UV light shows the dark band of histidase-F observed with the activity stain for histidase after 5 min. (B) This photograph under white light shows protein bands on a gel stained with Aniline Blue Black. (C) This photograph under UV light shows the bright (yellow) urocanase band observed with the activity stain for urocanase after 5 min. The sample on each portion of gel contained about 150 μg of protein. All three gel portions came from a single electrophoresis run. F, Histidase-F; S, histidase-S; U, urocanase.

ate. Fig. 1A is a photograph of a typical activity stain for histidase-F after 5 min incubation. Histidase-S (Fig. 1) was visible to the eye after 30–60 min but could not be easily photographed.

Both enzymes were revealed by longer incubation and careful observation. Fig. 2 is a photograph of an eosin-treated portion of gel which was incubated with L-histidine for 1 h. Both histidase bands and the urocanase band were visible. During 1 h incubation, the histidase converted histidine to urocanate (black background) which diffused through the gel. At the location of urocanase, this urocanate disappeared and a bright area appeared on the gel. Thus, it was possible to locate both enzymes with a single activity stain although the longer time required for a photograph makes the bands appear diffuse. The bands can be located precisely by observation at short intervals and by marking the gel with a slit when the bands first appear. When crude extract was heated (80°, 15 min) the urocanase and the histidase-S bands were no longer seen. Therefore, the electrophoresis was carried out at 4° as a precaution.

The gels were cut into strips, eluted and the enzymatic activity tested by

spectrophotometric and chromatographic methods. Once the enzymes were located by the activity stains on polyacrylamide gel, the bands from neighboring unstained gel were excised, eluted[6] and assayed on a spectrophotometer[5,6]. Activities (ΔA/5 min at 277 nm) of histidase-F, histidase-S and control bands were 0.120, 0.015, and 0.0, respectively, from a portion of gel containing approximately 150 μg protein. Excised bands of the enzymes were also tested for activity by paper chromatography[5].

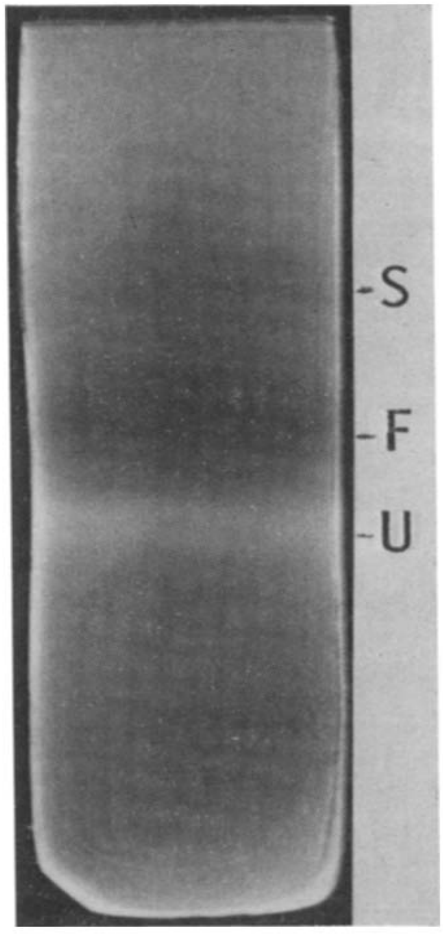

Fig. 2. Simultaneous detection of histidase and urocanase. This photograph under UV light shows the dark bands of histidase-F and histidase-S (slightly visible) and the bright band of urocanase (U) after 1 h incubation with L-histidine and eosin. The sample on this portion of gel contained 150 μg of protein.

Both methods confirmed that the activity stain detected protein bands with urocanase or histidase activity. Control protein bands which were negative to the activity stains had no enzyme activity by these methods. Analysis by either procedure showed that histidase-S has a much lower activity than histidase-F. Based on the relative intensity of the bands (F and S) after staining with Aniline Blue Black, it appears that the band histidase-S contains much less protein than histidase-F.

Discussion

This activity stain gives a rapid direct visible detection of active enzymes without the necessity of a photograph or other manipulations. It is possible to detect enzymes either in small amounts (approximately 3 μg of protein) or with low

activity by longer incubation with the substrate and it is covenient to compare directly samples in adjacent channels run under identical conditions. The evidence for two electrophoretically distinct forms of histidase in a crude extract is based on the two bands appearing after the histidase activity stain, the standard spectrophotometric assay of unstained bands after elution, and the conversion of histidine to urocanate when gel portions from unstained bands are incubated with histidine. There is a progressive conversion of histidine to a product which corresponds to urocanate in R_F value, color reaction to diazotized sulfanilic acid and UV quenching ability on paper chromatograms.

This investigation was sponsored by the Veterans Administration, Department of Medicine and Surgery, Research Service, Program 01/3795.1/69-04.

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Received October 27th, 1970

J. Chromatog., 54 (1971) 286–289

CHROM. 5044

A reaction for identification of Al^{3+}, Ga^{3+}, In^{3+}, and Tl^{+} separated by paper chromatography

Many reactions have been used for the identification of the periodic group III-A elements on paper chromatograms[1–4] but most of them are not common to the whole group of ions. In the present work, a reagent is proposed which forms colored complexes with Al^{3+}, Ga^{3+}, In^{3+} and Tl^{+}, allowing detection of all the above cations separated on a paper chromatogram.

Oxidized hematoxylin has been investigated by one of us[5] in the chromatographic identification of Ge(IV). The same reagent has now been found to give colored spots with all the ions mentioned above. Work involving the detection of group III-A elements in ores can be simplified by the use of this single detectant after separation by paper chromatography.