August 1972

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APPLIED MICROBIOLOGY, Aug. 1972, p. 283-285

Vol. 24, No. 2
Printed in U.S.A.

# Demonstration of Rhodanese Activity in Polyacrylamide Gels

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Received for publication 13 January 1972

Rhodanese activity from crude extracts of *Thiobacillus* sp. strain IV-85 was demonstrated in polyacrylamide gels after incubation in the reaction mixture by staining with dichloroindophenol in the presence of methylphenazonium methosulfate. The sensitivity of the staining system was found to be $8 \times 10^{-7}$ moles of sulfite.

Hunter and Markert (4) developed the technique of staining for specific enzymatic activities in starch gels after electrophoresis. This technique has been also applied to polyacrylamide gels. Recently, Shaw and Prasad (7) compiled the methods that were available for the specific staining of enzymes from various tissues and bacteria after electrophoresis. Guilbault, Kuan, and Cochran (3) described a procedure for the detection of partially purified bovine rhodanese in polyacrylamide gels.

In recent months, the enzyme rhodanese (thiosulfate:cyanide sulfur transferase, EC 2.8.1.1), which catalyzes the following reaction (equation 1):

$$S_2O_3^{2-} + CN^- \rightarrow SCN^- + SO_3^{2-} \quad (1)$$

has been the subject of many investigations. Burton and Akagi (1) described the rhodanese activity of *Desulfotomaculum*, and Murphy, Foulds, and Tilton (5) characterized the rhodanese activity of *Thiobacillus* sp. strain IV-85. In addition, Yoch and Lindstrom (10) surveyed the photosynthetic bacteria for rhodanese activity.

The observation of rhodanese activity in starch or polyacrylamide gels after electrophoresis would be of value in screening studies for taxonomic considerations and for following the activity of the enzyme after electrophoresis in other gel matrices. This investigation describes a technique for the qualitative estimation of rhodanese activity in a complex mixture of proteins after electrophoresis in polyacrylamide gels.

**Preparation of cell free extracts.** *Thiobacillus* sp. strain IV-85 was grown in thiosulfate salts broth as previously described (6). The cells were harvested by continuous-flow centrifugation, washed free of elemental sulfur, and stored in 0.067 M phosphate buffer, pH 8.6, at −20 C until used. The cells were lysed by freeze-thaw, and the whole cells and cellular debris were removed by centrifugation at 10,000 × *g* for 20 min. The supernatant fraction was treated with streptomycin sulfate (50 mg) and, after a 30-min incubation period at 4 C, the nucleic acid fraction and the membrane fraction were removed by centrifugation at 30,000 × *g* for 30 min. The 30 S 30 fraction was used as the source of crude cell-free extract for electrophoresis in polyacrylamide gels. The procedure for the purification of rhodanese from *Thiobacillus* sp. strain IV-85 will be described elsewhere (Murphy, Foulds, and Tilton, in preparation).

**Preparation of polyacrylamide gels.** Polyacrylamide gels (4.3%) and tris(hydroxymethyl)aminomethane(Tris)-glycine buffer (pH 8.6) were prepared according to the methods of Davis (2). In all experiments, the acrylamide columns were subjected to electrophoresis at 4 ma per column at 4 C. Protein samples were applied in duplicate. After electrophoresis, one of the duplicate columns was stained for protein with 1% Buffalo Black in 7% acetic acid, and the other gel was stained for rhodanese activity.

**Determination of rhodanese activity.** Rhodanese activity was assayed in the crude cell-free extracts according to the method of Sörbo (9), which measures the appearance of $SCN^-$ (equation 1). Rhodanese activity in polyacrylamide gels was determined by a modification of the method described by Smith and Lascelles (8), in which the formation of $SO_3^{2-}$ (equation 1) is linked to the reduction of dichloroindophenol (DCIP) in the presence of

methylphenazonium methosulfate (PMS) with the subsequent formation of $SO_4^{2-}$ (equation 2):

$$\underset{\text{(blue)}}{SO_3^{2-}} + \underset{\text{ox.}}{DCIP} \rightarrow \underset{\text{red.}}{DCIP} + \underset{\text{(colorless)}}{SO_4^{2-}} \quad (2)$$

After electrophoresis, the acrylamide columns were immersed in tubes containing $10^{-1}$ M $Na_2S_2O_3$ in 0.067 M phosphate buffer, *p*H 8.6, for 5 min. After incubation, an equal volume of $10^{-1}$ M KCN, in the above buffer, was added to initiate the reaction. The tubes were mixed and allowed to incubate at 30 C for 20 min. The reaction was stopped by the addition of 0.5 ml of 37% formaldehyde, and the gels were transferred to tubes containing 500 $\mu$moles of DCIP and 2.5 mg of PMS. The gel columns were developed for 8 min, removed, and placed in tubes containing 0.067 M phosphate buffer, *p*H 8.6, and then were photographed with a Polaroid MP-3 camera on type 48 film. Those areas which contained $SO_3^{2-}$ (equation 1) remained colorless (equation 2), whereas those areas of the gel which did not contain $SO_3^{2-}$ stained blue (equation 2). Those gels stained for rhodanese activity were photographed within 5 min after removal from the staining system.

As can be seen in Fig. 1, the electrophoresis of crude cell-free extracts of *Thiobacillus* sp. strain IV-85 in 4.3% polyacrylamide gels resulted in the separation of approximately 20 protein bands (Fig. 1a). The determination of the protein band associated with rhodanese activity was made by photographic comparison of the gel stained for protein (Fig. 1a) with the gel stained for rhodanese activity (Fig. 1b). Since the reduction of DCIP in the presence of PMS is specific for the sulfite ion, the appearance of the clear zone is indicative of rhodanese activity.

Rhodanese, from *Thiobacillus* sp. strain IV-85, has been partially purified in our laboratory by gel filtration on Sepharose 6B and diethylaminoethyl-cellulose ion exchange chromatography. The partially purified protein showed a single band after electrophoresis in 4.3% polyacrylamide gels (Fig. 1c). As can be seen in Fig. 1, the electrophoresis of purified rhodanese (c) directly corresponds to the lowest protein band in the gel stained for protein (a), and to the clear zone in the gel stained for rhodanese activity after electrophoresis of crude cell-free extracts (b). The sensitivity of the DCIP-PMS staining system was found to be $8 \times 10^{-7}$ moles of sulfite. The color reaction was found to be stable for 15 min, after which there was a decrease in coloration.

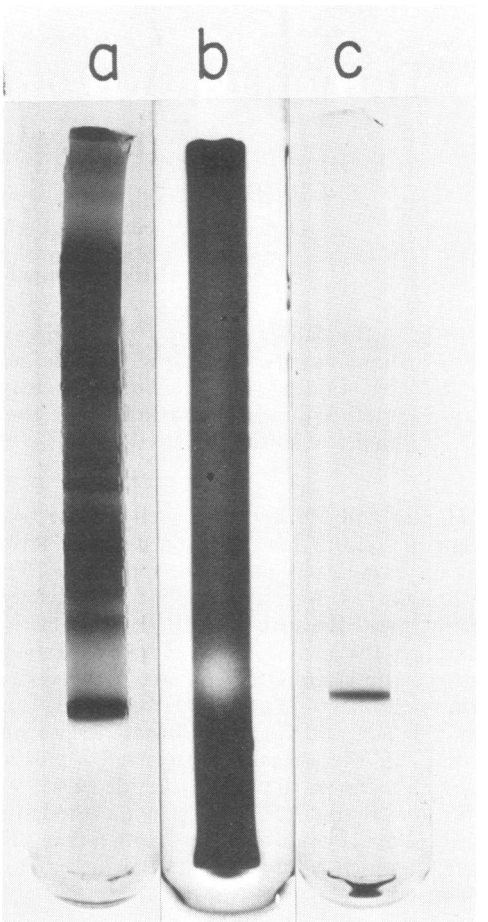


FIG. 1. *(a) 4.3% polyacrylamide column stained for total protein following electrophoresis of crude cell-free extracts; (b) 4.3% polyacrylamide column stained for rhodanese activity following electrophoresis of crude cell-free extracts; (c) 4.3% polyacrylamide column stained for total protein following electrophoresis of partially purified rhodanese from Thiobacillus sp. strain IV-85.*

Due to the specificity of the staining procedure for the presence of sulfite (equation 2), the detection of enzymes of either the sulfur oxidation pathway, or the sulfate reduction pathway, which produce sulfite, should be possible by the appropriate alteration of the reaction mixture.

This work was partially supported by a Public Health Service predoctoral traineeship RR-05678-03 to J.R.M.

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