An Electrophoretic Study of Purified Thrombin.* (24833)

WALTER G. LEVINE† AND OTTO W. NEUHAUS
(Introduced by W. H. Seegers)
Depts. of Physiology and Pharmacology and Physiological Chemistry, Wayne State University College of Medicine, Detroit, Mich.

In the present study, we investigated electrophoretic properties of purified bovine bioresin thrombin prepared as described by Seegers, Levine, and Shepard(1). The preparation had an average specific activity of 45,000 units/mg of tyrosine and 4100 units/mg of dry weight. In the analytical centrifuge the preparations showed a single symmetrical peak.

Materials and methods. Prothrombin was obtained from bovine plasma according to the methods of Seegers and co-workers(2,3). *Thrombin* was prepared by activating purified bovine prothrombin in the presence of lung thromboplastin and calcium ions(4). This material was then fractionated on the ion exchange column, the most active fractions were saved and finally obtained free of salt by methods previously described(1). Paper electrophoresis was performed with Spinco apparatus on Whatman No. 3 MM paper and with appropriate buffers of 0.1 ionic strength. Samples of .04% thrombin solution, .02 ml, were separated at room temperature using 120 volts for exactly 18 hours. The strips were then dried at 100°C and stained with 1% solution of bromphenol blue(5). After washing the strips in methanol, the color was intensified by ammonia fumes. The point of maximum color intensity was determined using a Photovolt densitometer. Samples of dextran,‡ .02 ml, were also included so that suitable corrections could be made for electroosmosis(5). Staining of dextran by bromphenol blue was usually insensitive even after 3 hours in the dye. The dextran area finally appeared as a light blue band; staining with periodic acid-Schiff reagent confirmed its identity.

Results are depicted in Fig. 1. Electrophoresis was performed at pH 5.0, 5.5, 6.0, 7.0, and 8.6, using acetate, phosphate, and veronal buffers, of 0.1 ionic strength, in appropriate pH ranges. At completion of electrophoresis, the pH of buffer was again tested to insure that no changes had occurred. In nearly every experiment, a single peak was obtained. However, no inferences may be made with respect to electrophoretic homogeneity of thrombin preparation because of diffusion effects and the relatively poor resolving power of filter paper electrophoresis.

To determine mobility and isoelectric point, it was necessary to correct migration for degree of electro-osmotic flow of buffer toward the cathode. All measurements of migration by thrombin were made using the dextran area as the actual origin. Thrombin mobility was calculated(5) to be 3.14×10^{-5} cm^2/volt/second at pH 8.6 which compares with 3.02×10^{-5} cm^2/volt/second, the value for β-globulin obtained by Kunkel and Tiselius(5). In an experiment using a sample of human serum, the purified thrombin migrated to the same degree as the β-globulins.

The isoelectric point (Fig. 1) was 5.6.

Discussion. From previous measurements of electrophoretic mobility of thrombin, the following values were obtained: biothrombin, 6.5×10^{-5}; citrate thrombin, 4.6×10^{-5} and 6.0×10^{-5} cm^2/volt/second respectively for the 2 major peaks observed(6). Isoelectric points of 4.1 and 4.7 were determined for citrate thrombin(6). We believe that the difference between previous values and the ones reported here, namely mobility of 3.14×10^{-5} cm^2/volt/second and an isoelectric point of 5.6, is great enough to be significant. Therefore, when biothrombin, our source material, is subjected to ion exchange chromatography, the electrophoretic properties of the

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† Present address: Dept. of Pharmacology, Albert Einstein College of Medicine, N. Y.

‡ Plavolex, a 6% solution of dextran, manufactured by R. K. Laros Co., Bethlehem, Pa.

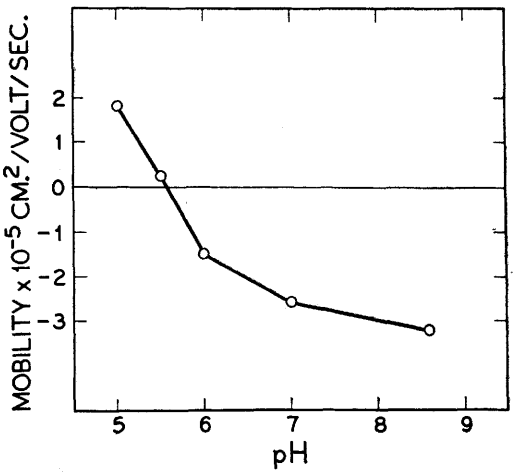

FIG. 1. Paper electrophoresis of purified thrombin with acetate, phosphate and veronal buffers, ionic strength 0.1.

material are altered as well as specific activity and ultracentrifugal properties already reported(1). In the citrate thrombin preparations previously studied it is likely that the results are in part the reflection of aggregate formation(7).

Summary. Electrophoresis on filter paper strips has been performed on purified thrombin prepared by recently developed technics of ion exchange chromatography. Mobility in veronal buffer, pH 8.6, ionic strength 0.1, was calculated to be 3.14 x 10^{-5} cm^2/volt/second. The isoelectric point was shown to be 5.6.

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Coupled Oxidative Phosphorylation in Crude Extracts of *Azotobacter agilis.* (24834)

EMMETT J. JOHNSON* AND C. E. CLIFTON

Depts. of Microbiology, University of Mississippi School of Medicine, Jackson, and Stanford University School of Medicine, Calif.

Oxidative phosphorylation in bacterial extracts is well authenticated(1-5), but details of this fundamental physiological mechanism are lacking. With exception of mycobacterial and corynebacterial systems of Brodie *et al.* (1), P/O ratios have been low compared to those theoretically possible, or are obtained with mammalian systems(6). It becomes desirable then to determine the influence, if any, of mode of preparation of the extract on P/O ratios. *Azotobacter agilis* (*A. vinelandii*) was employed as the test organism.

Materials and methods. Azotobacter agilis was grown with aeration in 3 L of Burk's nitrogen free mineral salts medium(7) containing 2% sucrose in 8-L pyrex glass bottle. The cells were harvested by centrifugation at 2°C and washed twice in cold distilled water. Intact cells, or disrupted ones, depending upon procedure employed, were suspended in 0.05 *M* "tris" (hydroxymethyl) aminomethane buffer, pH 8.0, containing 0.05 *M* $MgSO_4$; this solution is designated herein as TBS. Twenty-five μmoles ATP (N. B. Co.) were added to cell suspension or cell paste as stabilizer. *Sonic disintegration.* Two to 5 g of cells suspended in 5 ml of TBS were treated in 10 kc Raytheon magnetostrictive oscillator for 3 to 4 min. The homogenate was centrifuged 30 min at 20,000 X G and 2°C. The supernatant fluid (crude extract) was carefully decanted and enough phosphate buffer,

* Nat. Research Council Postdoctoral Fellow in Med. Sciences, Stanford 1957-1958.