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Abstract

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Hartman and Udenfriend (1969 Anal. Biochem. 30: 391) described a method for utilizing the mognerium salt of 1-anilino-8-naphthalene sulfonate (Eastman Organic Chemicals) as a mpid means for visualization of protein bonds in acrylamide gels. The advantages of this technique ore that the proteins within the gel ore not denatured and that the bands con be cut out, eluted, and assayed for enzyme activity in a few minutes' time. An attempt to obtain this compound showed that it was no longer available commercially, but Eastman Organic agreed to supply sampler of the free acid and its sodium salt, and to prepare a sample of the mognerium salt for evaluation.

The technique used war basically that described by the previous authors. Aqueous solutions (1 mg/ml) of each of the compounds were prepared and dilutions were mode in 0. 1 M potassium phosphate buffer, pH 6.8, to give final concentrations of 0.003%. The gels were prepored in the ORTEC pulsed-paver vertical gel electrophoresis apparatus, with sampler of 10–100 µg of protein per bond. The gels were immersed for approximately 1 min. in the stain ond then were viewed under a Mineral Light UV source. With this treatment, bond containing 50 µg of protein were easily visible when stained with the magnesium salt and were faintly visible with the sodium salt and the free acid.

By immersing the gel in 1 NHCl for a few seconds, rinsing in water, and restaining, we were able to detect 10-µg bonds with the mognerium salt, 20-µg bands with the sodium salt, and 50-µg bonds with the free acid. In all tests, the magnesium salt was markedly superior both to the sodium salt and to the free acid. The storage conditions for the reagents ore quite important. Both the stock solutions and the diluted stains were greatly inactivated by exposure to light for a few days. Storage in amber bottles with refrigeration prevented any detectable deterioration over a period of several weeks.

By combining this technique with modifications in the electrophoresis gel (described in the following communication), we were able to go from virtually no recovery' to a negligible loss of, enzyme activity. The mognerium salt, although not listed in the catalog, is now available from Eastman Organic Chemicals as catalogue number 10990. This material has already been recrystallized and requires no further treatment before use. (This research was sponsored by the U. S. Atomic Energy Commission under contract with Union Carbide Corporation and by the AEC Postdoctoral Fellowship Program of the Oak Ridge Associated Universities. • • • Biology Division, Oak -Ridge National Laboratory, Oak Ridge, Tennessee 37830.