Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis of N'-Dimethylaminonaphthalene-5-sulfonyl-Proteins: A Covalently Attached Fluorescent Label

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SUMMARY

Five reduced and alkylated proteins have been labeled with 1-dimethylaminonaphthalene-5-sulfonyl (DNS) chloride in sodium dodecyl sulfate (SDS) solution. The fluorescent DNS-proteins have been subjected to disc gel electrophoresis. Sensitivity of the method is such that a few micrograms of labeled protein can be easily revealed on conventional gels by UV light. By reduction of the gel diameter to 1 mm, a minimum of 0.05 μg of protein can be detected. The DNS group has at most a minor effect on the migration of the protein in the SDS-disc gel electrophoresis system.

The anionic detergent SDS is a powerful reagent for dissolving proteins and further, in conjunction with polyacrylamide disc gel electrophoresis, for determining approximate protein molecular weights.[1] Protein bands in disc gels are usually detected by radioactivity or by protein specific stains.[1,2] DNS chloride reacts with amines, thiols, imidazoles, and phenols in proteins to yield a fluorescent product which can be detected in very small quantity.[3,4] This report describes the use of the DNS group to label proteins before electrophoresis, thereby permitting direct visual observation of the protein at each step.

MATERIALS AND METHODS

Bovine γ globulin, egg white lysozyme, ovalbumin and bovine serum albumin were obtained from Sigma Chemical Company, St. Louis, Mo.

Reduction and alkylation were accomplished by slight modifications of published procedures.[1,2] 10 mg of protein were reduced in 10 ml of 1% SDS and 1% 2-mercaptoethanol for 3 hr at 37°. The solution was then made 0.3 M in iodoacetic acid, the pH adjusted to 8.3 with 2.0 M Tris buffer and incubation was continued for

1.5 hr at room temperature. The reduced and alkylated proteins were dialyzed overnight against three 1-liter volumes of 0.1% SDS at room temperature.

10 mg of DNS chloride and 0.5 ml of saturated sodium bicarbonate buffer were added to 3.0 ml of alkylated protein solution. The mixture was maintained overnight at room temperature. Undissolved DNS chloride was removed by centrifugation; the clear supernatant solution was dialyzed overnight against two 1-liter volumes of 0.0735 M Tris, pH 6.8, 0.1% SDS.

Gel electrophoresis followed the method of Laemmli.[5] Separation gels were 10% acrylamide, 0.27% N,N'-methylenebisacrylamide in 0.375 M Tris, pH 8.8, 0.1% SDS. Stacking gels were 3% acrylamide, 0.081% N,N'-methylenebisacrylamide in 0.125 M Tris, pH 6.8, 0.1% SDS. The electrode buffer was 0.025 M Tris, pH 8.3, 0.192 M glycine, and 0.1% SDS. Polymerization was catalyzed by addition of 0.005 ml of N,N,N',N'-tetramethylenediamine and 0.05 ml of 10% ammonium persulfate to each 10 ml of monomer solution. Separation gels were 9 x 0.5 cm, and stacking gels were 1 x 0.5 cm. Glycerol (1/10 volume) was added to each sample solution. Stacking gels were omitted and 1-3 μg of protein were used for molecular weight determinations.

Gels of 1-mm inside diameter were cast by pouring the monomer solution into a test tube containing the 1-mm tubes. Excess gel was removed after polymerization. Samples were applied with 0.015-inch inside diameter polyethylene tube attached to the needle of a 10 μl Hamilton syringe.

Fluorescent protein bands were revealed by longwave UV light and photographed in a Chromato-Vue box (Ultra-Violet Products, Inc.).

RESULTS AND DISCUSSION

As can be seen in Figure 1, a few micrograms of DNS-protein in 5-mm diameter gels contained sufficient fluorescence to be photographed. Bands could be detected by eye in the fourth gel. This sensitivity is comparable to that of amido black staining. It was possible to see 0.05 μg of protein in 1-mm gels.

The dependence of electrophoretic mobility on molecular weight for the proteins was not significantly affected by the DNS adduct. The five proteins migrated

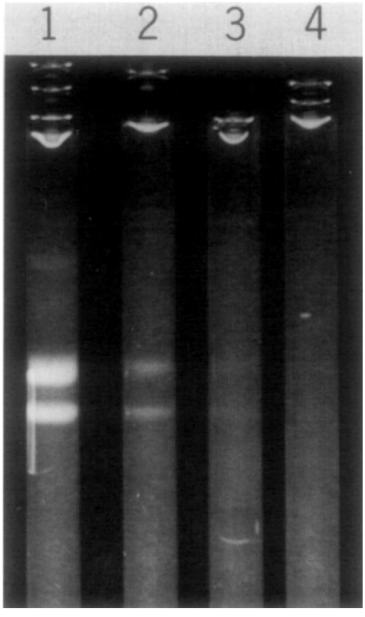

Fig. 1. Disc electrophoresis of reduced, alkylated, and DNS labeled bovine γ globulin as viewed under UV light. Total protein on each gel was (1) 30 μg, (2) 3 μg, (3) 0.6 μg, and (4) 0.3 μg. Migration was from top to bottom.

according to the equation log (mol wt) = -0.018 mobility (mm) +5.399, Standard Error of the estimate for log (mol wt) was $\pm$0.022. (Molecular weights were taken from reference 6.)

In order to detect slight changes in mobility due to DNS incorporation, each DNS-protein was compared with the unlabeled protein by means of split gel electrophoresis. There was no significant effect on electrophoretic mobility for the three smaller proteins, and further, no introduction of artifactual bands with any protein (Figure 2). An approximate 4% difference in migration occurred with BSA and the γ globulin heavy chain. The slight increase in mobility of the DNS-protein probably reflects a loss of positive charges due to the blocking of lysine residues.[7,8] SDS suppresses most of the potential effect of a protein's intrinsic charge.[1,2,6,8] This could account for the lack of visible heterogeneity in the DNS-protein, due to different DNS to protein ratios, which has been reported by others.[7]

These observations suggest that where unaltered protein is desired for chemical analysis, a small portion of a sample could be withdrawn, DNS labeled, and

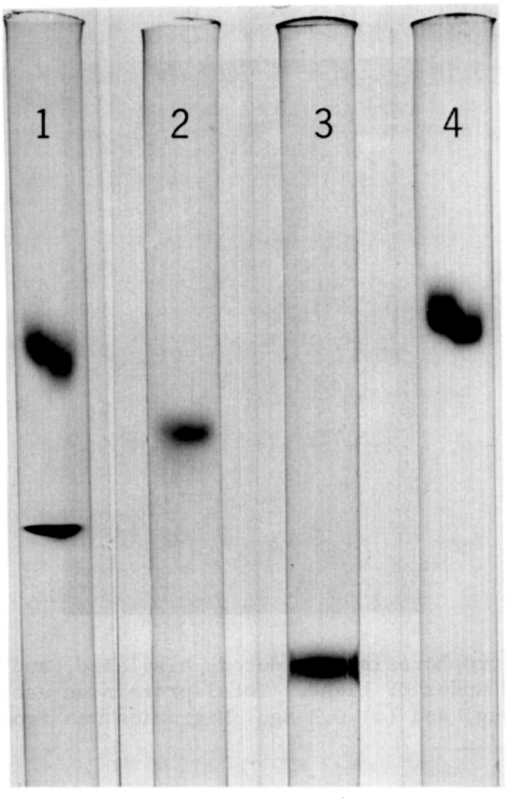

Fig. 2. Split gel electrophoresis of reduced and alkylated proteins with and without DNS addition. The right side of each gel contained the DNS-protein. Approximately 18 μg of each protein were applied. Electrophoresis, without a spacer gel, was for 3.5 hr at 3 ma/gel. Gels were fixed overnight in 20% sulfosalicylic acid, rinsed 3 hr in 7% acetic acid, stained 10 hr in 0.5% amido black in 7% acetic acid, and destained in 7% acetic acid. (1) Bovine γ globulin, (2) ovalbumin, (3) egg white lysozyme, and (4) bovine serum albumin. Migration was from top to bottom.

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a few per cent of DNS-protein returned to the original sample to provide a marker for each band. Alternatively, marker protein and sample could be kept separate on a split gel and the fluorescent portion discarded.

Labeling proteins with a fluorescent marker should increase the ease and speed of all subsequent manipulations. With small proteins requiring gels of relatively high acrylamide concentration, the fluorescent DNS-proteins will eliminate the requirement for very long staining and destaining times[8] and/or the significant loss es of protein during the staining and destaining steps.[9]

Isolation of protein from gel slices for further characterization should be facilitated by the fluorescent marker. One can observe directly the effects of

gel concentration, protein molecular weight, and history of the protein-gel mixture on the speed and efficiency with which protein is recovered. For example, the γ globulin light chain (23,500 mol wt) can be extracted from a 10% acrylamide gel slice (1 mm x 5 mm) in 10-20 hr with several 0.1% SDS washes. Under the same conditions, the heavy chain (50,000 mol wt) is poorly extracted.

A gel sufficiently strong to withstand handling during removal from the container and subsequent staining and destaining steps is not a requirement with the DNS-proteins. Therefore, it is possible to sharply reduce the gel diameter for molecular weight determinations if only limited protein is available.

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