Detection of Protein-Dodecyl Sulfate Complexes With Pinacryptol Yellow Following Electrophoretic Separation

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Summary. A rapid technique for the location of proteins separated electrophoretically in the presence of sodium dodecyl sulfate in just the sample solution is described. This procedure involved the use of pinacryptol yellow to locate the protein-dodecyl sulfate complex in the polyacrylamide gel that previously had been subjected to electrophoresis. With pinacryptol yellow as a means of visualization, the time required for the detection of the protein-dodecyl sulfate bands was greatly reduced due to the elimination of the lengthy staining and destaining procedures.

Introduction

An essential part of molecular weight determinations of proteins in the presence of SDS* is the location of the protein band in the gel. Most of the methods presently employed involve the use of a biological stain such as Amido Black or Coomassie Blue (1-4). Although these stains do provide a simple and effective way to locate the bands, they are time consuming due to the lengthy staining and destaining procedures.

There are techniques which eliminate these lengthy procedures. By labeling the protein with a fluorescent compound prior to electrophoresis, the resulting protein-fluorophor can be located with an appropriate excitation source. Some of the compounds which have been used to label proteins are fluorescamine (5,6), 1-dimethylaminonaphthalene-5-sulfonyl chloride (7), anilinonaphthalene sulfonate (8,9) and dansyl chloride (10).

With the innovation of using SDS in just the sample solution (11), methods used for the visualization of surfactants like SDS can now be employed to locate

*Abbreviation for sodium dodecyl sulfate.

the protein-SDS complex in the gel. One such method involves the use of pinacryptol yellow which reacts with SDS to form a yellowish orange fluorescent precipitate (12,13).

Materials

The following proteins were obtained from Sigma Chemical Company: catalase, egg albumin, pepsin, α-chymotrypsinogen-A, myoglobin, hemoglobin, lysozyme and cytochrome-C. Serum albumin and γ-globulin (H and L chain) were supplied by Nutritional Biochemical Company.

The gels were prepared with Cyanogum (95% acrylamide and 5% N,N-methylene-bisacrylamide) obtained from Nutritional Biochemical Co. The catalysts used for polymerization were N, N, N',N'-tetramethylenediamine (TMED) obtained from Bio-Rad Laboratories and ammonium persulfate (AP) obtained from Fisher. High purity SDS was purchased from Pierce Chemical Co.; Brilliant Blue R from Sigma; $NaH_2PO_4 \cdot H_2O$ and $Na_2HPO_4 \cdot 7H_2O$ from Fisher. Pinacryptol yellow was supplied by K and K Laboratories. The U.V. source was purchased from Ultra-Violet Products, Inc. The gels were scanned with an Aminco-Bowman spectrophotofluorometer equipped with a thin-layer scanner.

Methods

Electrophoresis was performed according to a procedure based on the one described by Weber and Osborn (1) with a modification we have reported previously (11). The proteins were dissolved at a concentration of 0.5 mg/ml or 1 mg/ml in a solution containing SDS (0.1%, 0.2% or 0.3%), 0.01 M phosphate at a pH = 7.2 and 0.1% β-mercaptoethanol. These solutions were stored overnight at room temperature. The buffer in the electrode compartments and in the gels was 0.1 M phosphate at a pH = 7.2. Gels were prepared with Cyanogum (5%, 6%, 7% or 8%), 0.1% AP and 0.1% TMED. The sample solution applied to the top of each gel contained 20μl of the protein solution (10μg - 20μg), 25μl of the SDS solution previously used to dissolve the protein, 5μl of β-mercaptoethanol, 15μl of 0.25% bromphenol blue and 1 drop of glycerol. A constant current of 6 ma per tube was applied for $3\frac{1}{2}$ to 4 hours. The gels were removed from the tubes and placed in

an aqueous solution of 0.01% pinacryptol yellow for approximately 30 minutes. Then the gels were taken out of the pinacryptol yellow solution, rinsed with distilled water, placed under the U.V. light and the migration distances of the observed bands were measured.

The emission profiles of the resulting protein-SDS-pinacryptol yellow and SDS-pinacryptol yellow adducts were obtained by placing the gel in a 20cm quartz tube and positioning this tube on the motor-driven scanning frame within the thin-layer scanner unit. The excitation monochromator was set at 406nm with the emission set at 544 nm. The slit program was 5-4-0.5-5-5 and the meter multiplier dial on the photomultiplier microphotometer was set at 0.01.

Results

Using pinacryptol yellow as a means of detection, fluorescent bands were observed for all the proteins within 30 minutes after electrophoretic separation was completed. Depending on the range of molecular weights of interest the % SDS and the % Cyanogum were adjusted in order to obtain intense reproducible bands within this molecular weight range. The reason for this adjustment was that in addition to the protein-SDS complex, bands due to the excess SDS present in the sample solution were observed. The appearance of several SDS bands was consistent with the observations made by Bodenmiller (14). These SDS bands presented problems in the fact that their relative mobilities were similar to the bands of several of the proteins. To alleviate this problem, the % SDS and the % Cyanogum were varied since the mobility of the SDS was largely dependent on the amount present and the mobility of the protein-SDS complex was dependent on the gel concentration. Therefore by optimizing the % SDS and the % Cyanogum, the overlap between the protein-SDS bands and the SDS bands was decreased. A summary of several of the conditions employed and the protein bands observed under these conditions are given in Table I. The results of these variations in experimental conditions indicate that with 0.2% SDS and 7% Cyanogum bands were observed over the widest range of molecular weights. The other conditions listed in Table I were useful depending on the molecular weight of the protein.

Protein	5% Gels and 0.1% SDS	6% Gels and 0.3% SDS	7% Gels and 0.2% SDS	8% Gels and 0.3% SDS
serum albumin	x	x	x	-
catalase	x	x	x	-
γ-globulin (H-chain)	x	x	x	-
egg albumin	x	x	x	x
pepsin	-	-	x	x
α-chymohypsinogen-A	-	x	x	x
γ-globulin (L-chain)	-	x	x	-
myoglobin	-	x	x	x
hemoglobin	-	-	x	x
lysozyme	-	-	x	x
cytochrome-C	-	-	x	x

Table I: Summary of Experimental Conditions

x observable band

- sporadic appearance of band

With 8% gels there were no observable bands for the higher molecular weight proteins when electrophoresis was performed for longer than 4 hours. A possible reason for this is that the protein-SDS complex dissociates with time. However evidence obtained does not support this. The gels which had no observable bands for the high molecular weight proteins were placed in a Coomassie Blue solution. Following the staining and destaining procedure, bands were observed with relative mobilities characteristic of the molecular weight of protein. Also under other conditions such as 0.1% SDS with 5% gels, fluorescent bands were observed for these proteins without any reduction in their relative mobilities even after a five-hour run. Another possible explanation is that the formation of the protein-SDS-pinacryptol yellow precipitate depends on the amount of SDS in the gel. The results tend to support this since there was a fluorescent background due to excess SDS at the location of the

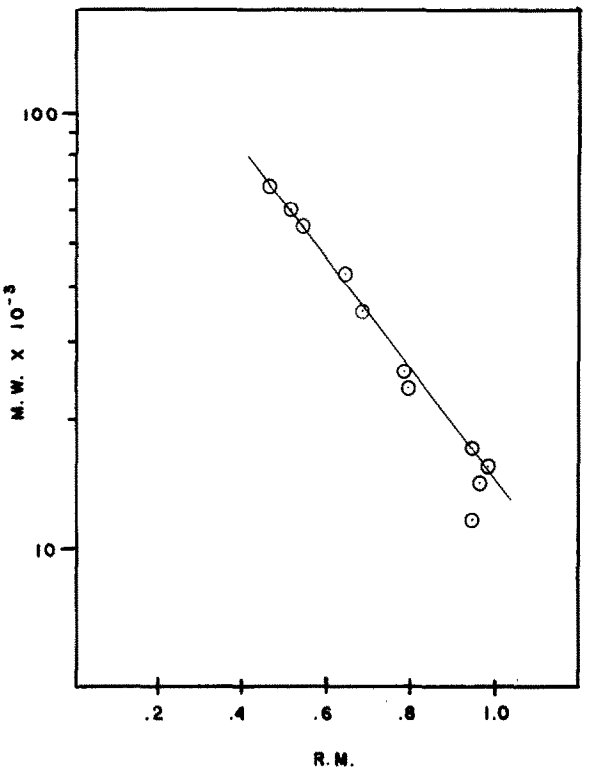

Figure 1: Relationship between the relative mobility (R.M.) and the molecular weight (M.W.) of the proteins with 0.2% SDS in the sample solution and 7% Cyanogum gels detected with pinacryptol yellow.

protein bands for all the proteins under any of the conditions employed.

Based on the results obtained with 0.2% SDS and 7% gels, a calibration curve was constructed with the calculated relative mobilities of each protein plotted against their respective molecular weights. The resulting relationship is illustrated in Figure 1. These calculated relative mobilities, based on an average of 12 separate runs were as reproducible as those obtained with Coomassie Blue detection. In terms of sensitivity, the observed bands under these specific conditions were generally not as intense as those observed with Coomassie Blue. However it appeared that the intensity of a particular protein-SDS band depended on the experimental conditions employed.

Using this method of detection, the molecular weight of malate dehydrogenase

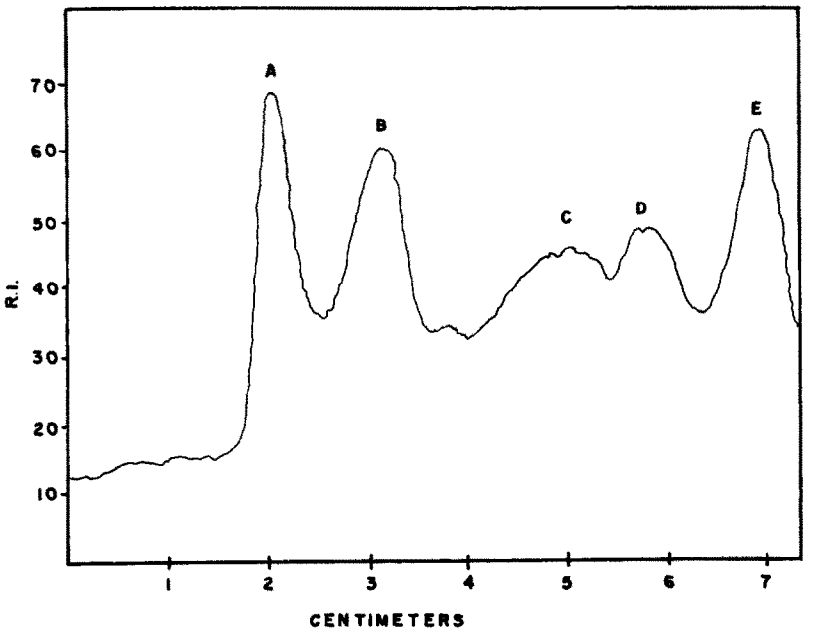

Figure 2: Separation profile of a mixture of proteins (20μg of each one) with the relative intensity (R. I.) of the fluorescent pinacryptol yellow precipitate as a function of the location in the gel. The conditions employed were 0.3% SDS and 7% Cyanogum. The proteins were serum albumin (A), egg albumin (B) and myoglobin (D). Peaks C and E were due to the excess SDS.

isolated by Dr. Eugene M. Gregory‡, was rapidly verified. Both 0.3% SDS with 8% gels and 0.2% SDS with 7% gels were used.

A simple detection technique has been described which greatly reduces the time required for a complete molecular weight determination without any alterations in the relative mobilities of the protein-SDS complex. An added advantage is that these bands are fluorescent which permits scanning of the gels with a fluorometer as illustrated in Figure 2. At this point it appears that in order to attain this reduced analysis time, a sacrifice must be made in terms of sensitivity.

‡Private consultation in this laboratory.

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