Molecular Weight Determinations of Proteins by Polyacrylamide Gel Electrophoresis with Sodium Dodecyl Sulfate in Just the Sample Solution

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Summary. This report describes a simple modification of the procedure for SDS polyacrylamide gel electrophoresis by using SDS in the sample solution and eliminating its use in the gel and the electrode compartments. The results obtained were comparable to those with SDS in the entire system. The log molecular weight-relative mobility plots for a variety of proteins, the reproducibility and the appearance of the bands such as their intentisy and sharpness were very similar. Therefore SDS in the entire system is not necessary to determine the molecular weight of a protein.

Introduction

Molecular weight determinations of proteins by polyacrylamide gel electrophoresis in the presence of SDS is a widely used technique (1-14). This method which was first employed by Shapiro, Vinuela and Maizel is based on the fact that the relative mobility of a protein-SDS complex in a polyacrylamide gel medium is related to its molecular weight (1). Basically the procedure followed has been one of using SDS throughout the entire system which includes the sample solution, the gel and the buffers in the electrode compartments. It is the purpose of this report to illustrate that SDS is not required in the gel or the buffer in the electrode compartments but only in the sample solution.

Materials

The proteins used are listed in Table I. The gels were prepared from Cyanogum 41 (95% acrylamide and 5% N,N-methylenebisacrylamide) purchased

from Nutritional Biochemical Co. High purity SDS was obtained from Pierce Chemical Co; Brilliant Blue R from Sigma; $NaH_2PO_4 \cdot H_2O$ from J. F. Baker Chemical Co.; Bromphenol Blue, ammonium persulfate and $Na_2HPO_4 \cdot 7H_2O$ from Fisher; TEMED from Bio-Rad Laboratories. All other chemicals were reagent grade.

Method

The procedure used was based on the one described by Weber and Osborn (4). The individual proteins were dissolved in .01 M phosphate buffer at pH = 7.2 containing SDS (0.1% or 0.3%) and 0.1% β-mercaptoethanol. These protein solutions were stored overnight at room temperature. Buffer for the upper and lower electrode compartments was 0.1 M phosphate at a pH = 7.2 with no addition of SDS. Solutions used to form the polyacrylamide gel contained the appropriate amount of Cyanogum 41 to obtain a 5% concentration of acrylamide, 0.1% ammonium persulfate and 0.1% TEMED. Again no SDS was used for the gel preparation. This gel solution was placed in glass tubes of 10 cm in length with an inside diameter of 5 mm and then a drop of water was layered on the top of the gel solution. After approximately 15 minutes, gelation occurred. The water was removed and a sample solution was placed on the top of the gel. This sample solution contained 20μl of the protein solution, 20μl of the phosphate buffer which was previously used to dissolve the proteins, 10μl of 0.25% Bromphenol Blue, 5μl of β-mercaptoethanol and 1 drop of glycerol. Electrophoresis was performed at a constant current of 6 ma per tube for approximately 3-1/2 hours. Subsequent staining and destaining were carried out in the exact same manner as Weber and Osborn. (4)

Results

Using the above described procedure with SDS in just the sample solution, bands were observed for all 15 proteins listed in Table I. The calculated relative mobilities of these proteins, based on an average of approximately 15 separate runs for each protein, were plotted against their respective

Table I

Protein	Source	Molecular Weight	Reference
Serum Albumin trimer	N.B.C.	204,000	a
Serum Albumin dimer	N.B.C.	136,000	a
Ovalbumin dimer	Sigma	86,000	b
Serum Albumin monomer	N.B.C.	68,000	4
Catalase	Sigma	60,000	4
γ-Globulin, H chain	N.B.C.	50,000	4
Ovalbumin momer	Sigma	43,000	4
Pepsin	Sigma	35,000	4
α-Chymotrypsinogen-A	Sigma	25,700	4
γ-Globulin, L chain	Sigma	23,500	4
Myoglobin	Sigma	17,200	4
Hemoglobin	Sigma	15,500	4
Lysozyme	Sigma	14,400	4
Cytochrome-C	Sigma	11,700	4
Insulin	Sigma	5,700	2

a Calculated assuming multimers of serum albumin

b Calculated assuming dimer of ovalbumin

molecular weights and the results are shown in Figure 1 and Figure 2 for a 0.3% and 0.1% concentration of SDS respectively. For each concentration the plot was linear over a range characteristic of the gel composition. The observed bands for all the proteins were generally intense and well-defined for 0.01 mg of protein which was the amount used for all the proteins. In regard to the reproducibility of this method the relative deviation of the calculated average relative mobilities was less than 5%. These results are comparable to those obtained with SDS in the entire system as reported by other workers (1-14) and also by the results we obtained at a 0.3% and 0.1% concentration of SDS in the entire system (Figure 3 and Figure 4). The linear range of the relationship between log molecular weight and relative

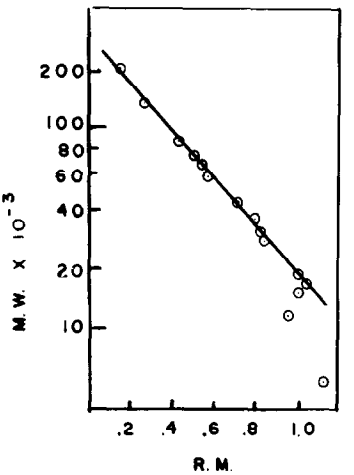

Figure 1
Electrophoretic mobility as a function of molecular weight of the proteins with 0.3% SDS in sample solution only.

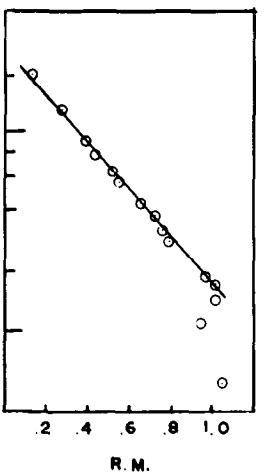

Figure 2
Electrophoretic mobility as a function of molecular weight of the proteins with 0.1% SDS in sample solution only.

mobility, the intensity and sharpness of the bands and the reproducibility of both methods were quite similar. Therefore with this method less time was required to prepare the buffer solutions for the gels and the electrode compartments and the amount of SDS was greatly reduced without any sacrifice of the linear range, sensitivity and reproducibility.

Another important aspect of this method was that it illustrated the stability of the protein-SDS complex. It has been proposed that the interaction between the proteins and SDS is hydrophobic in nature (15). It was apparent from our work that this interaction was stable under a voltage gradient of approximately 4 volts per cm and a concentration gradient since there was no SDS in the gel or electrode compartments. Even the basic proteins such as lysozyme, cytochrome-C and α-chymotrypsinogen-A produced intense sharp bands characteristic of a negatively charged protein-SDS complex even though one would have expected these proteins to be the least stable because of their native positive charge.

Using this method a new detection system was employed which reduced

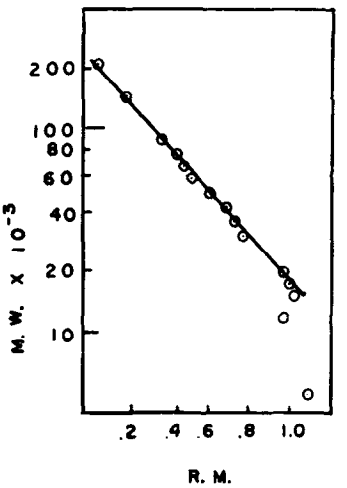

Figure 3

Electrophoretic mobility as a function of molecular weight of the proteins with 0.3% SDS in the entire system.

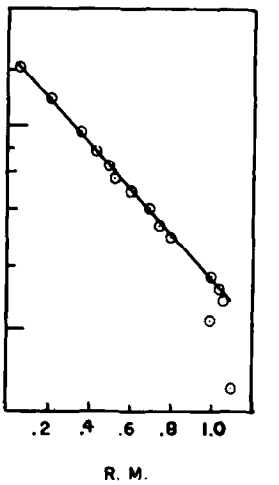

Figure 4

Electrophoretic mobility as a function of molecular weight of the proteins with 0.1% SDS in the entire system.

the lengthy staining procedure and eliminated the destaining procedure used with Coomassie Blue as the staining dye. Since the only SDS in the system was either bound to the proteins or remained in the sample solution after a certain amount reacted with the protein, pinacryptol yellow was used to locate the SDS which in turn determined the location of the protein-SDS complex. Several proteins were run in this manner and the characteristic yellow bands due to the reaction of the SDS with the pinacryptol were observed in approximately 1/2 hour after the gel was immersed in the pinacryptol yellow solution. The calculated relative mobilities for these proteins corresponded to those obtained with the Coomassie Blue staining procedure. In addition to the bands observed for the protein-SDS complexes, a band due to the remaining SDS not bound to protein in the sample solution was observed. However this band did not interfere with the protein-SDS bands. When pinacryptol yellow was used with gels that were prepared with SDS, a considerable amount of background due to the SDS throughout the gel was observed. Therefore it was very difficult to locate the bands. When

gels which were prepared without SDS were placed in the pinacryptol yellow solution, the bands were easily located because the background was greatly reduced. These are only preliminary results for a few proteins (serum albumin, ovalbumin, pepsin, myoglobin and hemoglobin). More work is now being done with this detection method and will be reported at a future time.

The general procedure of Weber and Osborn was followed in our experimentation and no attempt was made to make any major alterations in their procedure except for the removal of SDS from the gels and the electrode compartments. Work has been published where modifications such as an increase in the acrylamide concentration (2,7), the use of the disc electrophoretic technique (5) and the addition of urea to the system (7) were used in order to improve separation or extend the linear range of molecular weights. No major problem can be foreseen in adapting the method described in this report to these other techniques.

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