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Note

Fluorescence micro disc electrophoresis in sodium dodecyl sulfate

A simple and sensitive method applicable for routine laboratory analysis

W.Y. CHAN, T. SEALE and O M. RENNERT*

Department of Pediatrics and Department of Biochemistry and Molecular Biology, Oklahoma University Health Sciences Center, Oklahoma City, Okla. 73190 (U.S.A.)

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Sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis has become a major analytical tool in protein biochemistry since its first description in 1967 [1]. However, traditional SDS polyacrylamide gel electrophoresis suffers from the disadvantage of requiring a relatively large quantity (microgram levels) of protein to give a detectable band [2]. Numerous investigators have attempted by the application of two approaches to increase the sensitivity of this method.

Diminution of column dimensions increases the sensitivity by reducing the cross-sectional area of the gel. A variety of micro-gel systems have been developed by employing capillary tube electrophoresis [3—6]. These methods involve tedious handling procedures [3—6], special apparatus [5, 6] or loss of resolution and reproducibility [5, 6]. Such methods, however, are successful in increasing sensitivity to the nanogram protein concentration range.

Conventional staining of protein bands with Coomassie brilliant blue or amido black have several inherent disadvantages. The requirements for fixation, staining, and destaining are time consuming. To be stained effectively the gels need to be removed from the glass columns. Such dye staining techniques require relatively high protein concentration and the sensitivity decreases with decreasing molecular weight.

Quantitation of these dye staining methods depends on absorption of light. Given a fluorophor with a good quantum yield, fluorescence allows an increase in sensitivity of about 10^3 in comparison to absorption [7]. Thus, fluorescence

*To whom correspondence should be addressed.

methods were introduced into polyacrylamide gel electrophoresis [8—13]. The most popularly used fluorescent labels are fluorescamine [8—10] and 1-dimethylaminonaphthalene 5-sulfonyl chloride (dansyl chloride) [11—13]. The fluorescent methods are sensitive down to the nanogram range and are especially useful when working with low-molecular-weight proteins. Dansylation of peptides has no effect on the linear relationship between mobility and molecular weight over a molecular weight range of 1000—12,000 [13].

The present communication describes a combination of the micro-gel approach with the dansyl chloride fluorescent labelling in SDS polyacrylamide gel electrophoresis. This technique is more sensitive than any of the reported SDS polyacrylamide gel electrophoresis methods and is suitable for routine use in ordinary laboratories.

MATERIALS AND METHODS

Acrylamide, N,N'-methylenebisacrylamide (Bis), ammonium persulfate, and N,N,N'N'-tetramethylethylenediamine (TEMED) were purchased from Bio-Rad Labs. (Richmond, Calif., U.S.A.). SDS, urea, tris(hydroxymethyl)aminomethane (Tris), tris(hydroxymethyl)aminomethane acetate (Tris acetate), potassium ferricyanide, B-mercaptoethanol and dansyl chloride were supplied by Sigma (St. Louis, Mo., U.S.A.). The column coat Dri-Film SC-87 (surfasil) was supplied by Pierce (Rockford, Ill., U.S.A.), acetone by Aldrich (Milwaukee, Wisc., U.S.A.) and Dichrol acid cleaning solution by Scientific Products (State College, Pa., U.S.A.). The protein standards ovalbumin, soybean trypsin inhibitor, lysozyme (egg white), cytochrome *c* (horse heart Type III), ribonuclease A (bovine pancreas), lima bean trypsin inhibitor, polylysine (<3000 daltons polymer), angiotensin II, oxytocin and gramicidin S (*Bacillus brevis*) were from Sigma and insulin (bovine pancreas), adrenocorticotrophic hormone (porcine) (ACTH), glucagon, bacitracin and methionine-enkephalin were from Calbiochem (Los Angeles, Calif., U.S.A.).

Procedures for dansylation of protein and preparation of gels were similar to that described by Kato and Sasaki [13] except that the dansylation process was 45 min instead of 15 min. 100-μl disposable microcapillary pipets (1 × 128 mm) (Dade Diagnostic) were acid cleaned and coated with a 0.5% solution of Dri-Film SC-87 in acetone. Micro gels were made by dipping five clean capillary pipets into a clean standard bore glass electrophoresis gel tube (6 × 130 mm) (Bio-Rad Labs.) sealed at one end with parafilm and filled with gel solution. Water was layered on top of the gel to maintain a level surface and the gel was allowed to polymerize for about 30 min. The micro gel tubes could be removed from the standard bore glass electrophoresis gel tube using a glass rod.

The electrophoresis buffer was prepared by tenfold dilution of 1 *M* tris acetate buffer, 1% SDS, pH 8.2. The gel surface was washed several times with the electrophoresis buffer before use. The gel tubes were attached to the upper reservoir of a Bio-Rad Model 155 gel electrophoresis cell by using the stoppers of ordinary vacutainer tubes. The electrophoresis cell was then filled with buffer as recommended [14]. Care was taken that no air bubbles were trapped at either end of the gel. Ten μl of the dansyl-protein solutions were layered

onto the gels using an ordinary 10-μl Hamilton microliter syringe No. 701. Electrophoresis was performed with a LKB 2103 power supply at constant current of 0.15—0.25 mA/gel. Mobility of the bands during electrophoresis can be monitored at 366 nm using a UV lamp (UV SL-25, Ultraviolet Products).

RESULTS

A typical run with 0.25 mA/gel took 6—7 h for 12.5% SDS gel and 8 h for 15.0% SDS gel. The electrophoretic patterns of authentic standard peptides run in 12.5% and 15.0% SDS gel were similar to those reported previously [12]. The brightest and fastest moving band in each gel was 1-dimethylaminonaphthalene 5-sulfonic acid (dansyl-OH) formed during the dansylation reaction [12]. One inherent shortcoming of reported micro gel methods is the relatively rapid diffusion of the protein bands after electrophoresis. However, with the present method, even with the band-spreading effect, dansylated polypeptides including cytochrome *c*, lima bean trypsin inhibitor, ACTH, bacitracin and angiotensin II gave quite discrete bands. The mobility of dansylated protein in the gel was not affected by mixing with other dansylated species as shown by the fact that the mobility of dansylated cytochrome *c* was not altered by mixing with lima bean trypsin inhibitor. The optimum amount of peptide applied was found to be 50 pmole. It was demonstrated based upon the brightness of the dansylated peptide bands that 25 pmole of certain polypeptide gave clearly distinct resolution at 366 nm.

Relative mobility of the dansylated peptide was calculated by dividing the migration distance of the dansylated peptide by that of dansyl-OH. Table I pre-

TABLE I

COMPARISON OF THE RELATIVE MOBILITIES OF PEPTIDE STANDARDS IN 12.5% AND 15.0% SDS MICRO GELS

The relative mobility was the average of five runs ± S.D.

Peptide standards	Molecular weight (reference)		Relative mobility	
			12.5% SDS	15.0% SDS
Ovalbumin	44,000	[16]	–	0.14 ± 0.01
Soybean trypsin inhibitor	21,600	[17]	0.145 ± 0.005	0.195 ± 0.005
Lysozyme	13,930	[16]	0.235 ± 0.005	0.255 ± 0.005
Ribonuclease A	13,690	[18]	0.185 ± 0.004	0.245 ± 0.006
Cytochrome *c*	12,400	[18]	0.18 ± 0.01	0.280 ± 0.003
Lima bean trypsin inhibitor	8,400	[18]	0.23 ± 0.01	0.320 ± 0.008
Insulin	5,782	[18]	0.25 ± 0.01	0.405 ± 0.005
			0.37 ± 0.01	0.505 ± 0.005
ACTH	4,600	[19]	0.250 ± 0.002	0.350 ± 0.004
Glucagon	3,483	[18]	0.223 ± 0.003	0.395 ± 0.002
Polylysine	3,000	[20]	0.22 ± 0.01	0.435 ± 0.015
Bacitracin	1,411	[19]	0.309 ± 0.005	0.47 ± 0.01
Angiotensin II	1,178	[20]	0.250 ± 0.009	0.48 ± 0.01
Oxytocin	1,000	[20]	n.d.*	n.d.*
Gramicidin S	1,000	[20]	0.340 ± 0.002	0.520 ± 0.009
Methionine-enkephalin	645	[20]	–	0.590 ± 0.008

*n.d. = not detectable.

sents the peptides and their relative mobilities in both 12.5% and 15.0% SDS gel. Relative mobilities are presented as the average of five independent experiments plus or minus standard deviations. Dansylated oxytocin was identified as a faint smear or was not detectable in either the 12.5% or 15.0% SDS gels at quantities above 100 pmole. Insulin consistently gave two bands in both gel concentrations. This was in agreement with observations reported previously [13, 15].

Fig. 1 is a plot of the logarithm of the molecular weight (MW) of the peptide standards against their relative mobilities in 12.5% SDS gel. A linear plot was obtained from soybean trypsin inhibitor (MW 21,700) to ACTH (MW 4600). The plot was close to linearity for peptides of smaller molecular weights such as bacitracin, angiotensin II and gramicidin S. There were deviations from linearity in the case of lysozyme, glucagon and polylysine. These may be due to the intrinsic charge and shape of the polypeptides as suggested by Kato and Sasaki [13]. Fig. 2 is a plot of the logarithm of molecular weight against relative mobility of the peptide standards in 15.0% SDS gel. The plot was linear from ribonuclease A (MW 12,600) to bacitracin (MW 1411). Slight deviation from linearity was observed for molecules such as soybean trypsin inhibitor, lysozyme, cytochrome *c*, angiotensin II and gramicidin S. Despite the deviation of

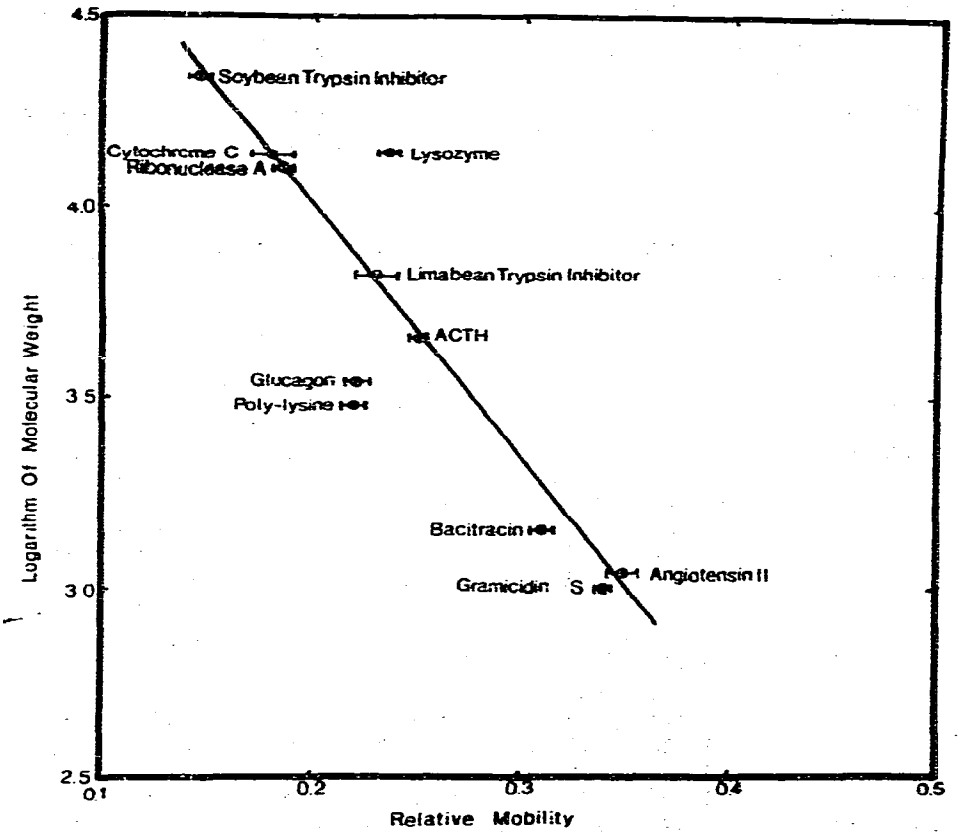

Fig. 1. Plot of the logarithm of the molecular weight of peptides versus their relative electrophoretic mobilities on 12.5% SDS micro polyacrylamide gel.

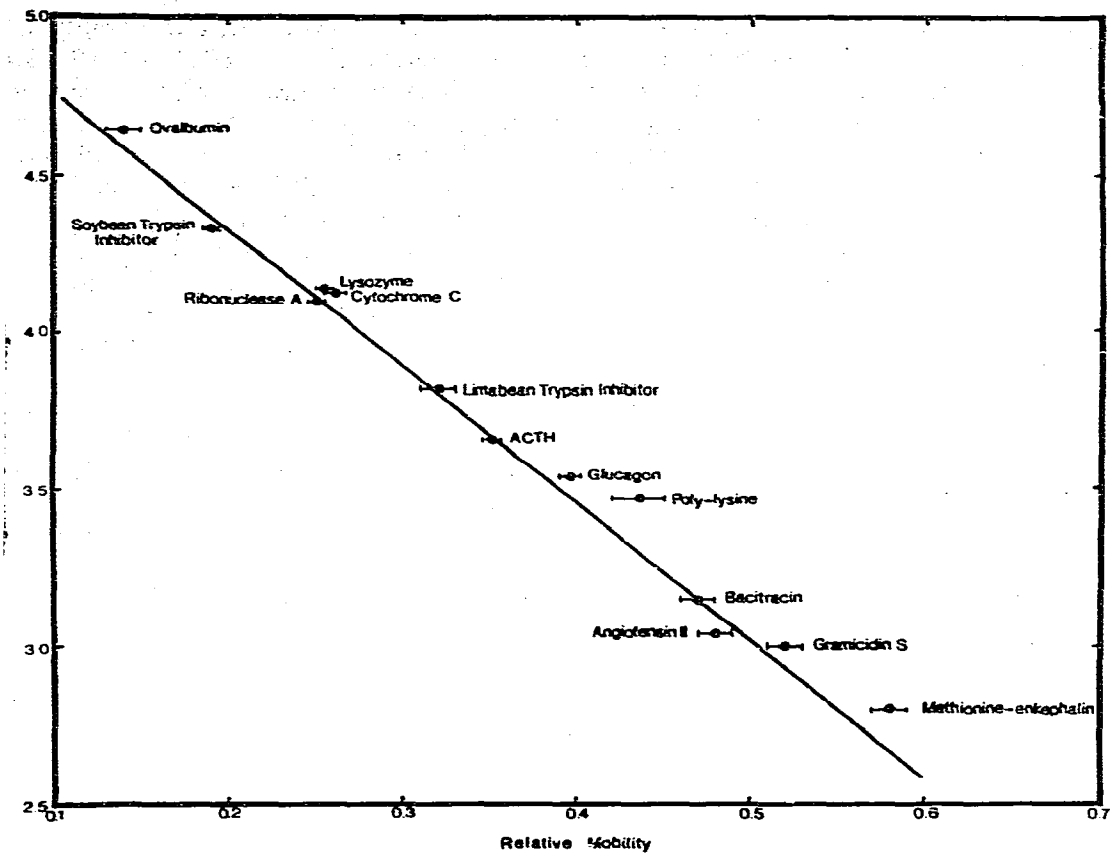

Fig. 2. Plot of the logarithm of the molecular weight of peptides versus their relative electrophoretic mobilities on 15.0% SDS micro polyacrylamide gel.

some standards, e.g. ovalbumin, glucagon, polylysine, methionine-enkephalin, from linearity, the 15.0% SDS gel gave a good linear relationship between molecular weight and relative mobility especially in the molecular weight range of 12,600—1400. Comparing the behavior of dansylated peptides in 12.5% and 15.0% gels, it is obvious that the 12.5% SDS gel is better for higher molecular weight peptides ranging from 22,00—4600 and the 15.0% SDS gel is better for lower-molecular-weight peptides.

DISCUSSION

In polyacrylamide gel electrophoresis, the gel tubes were usually siliconized so that the gel column would be removed easily following electrophoresis. This step is especially important in micro gel electrophoresis. Air bubbles easily formed along walls of untreated capillary tubes during gel polymerization. A 0.5% solution of Dri-Film SC-87 in acetone was found to be effective in preventing bubble formation.

Traditional polyacrylamide gel electrophoresis in concentrated gel (12.5%

SDS gel) containing 0.1% SDS and 8 *M* urea was reported to have the ability to fractionate peptides with molecular weights ranging from 1000 to 12,000 [13—15]. However, due to the difference in internal diameter of the conventional gel tubes (standard bore glass electrophoresis gel tube 6 × 130 mm) and the micro-capillary pipets (1 × 128 mm), the 12.5% SDS micro gel is capable of fractionating molecules with weight between 22,000—4600 with a linear relationship between relative mobility and logarithm of molecular weight. Fig. 2 shows that in 15.0% SDS micro gel, a linear plot of the logarithm of molecular weight versus relative mobility was obtained for peptides of molecular weights 12,600—1400. Thus, the fractionation capability of the 15.0% SDS micro gel is comparable with that of the conventional 12.5% SDS gel reported previously [13].

As noted in the results, most peptide standards gave distinct visible bands at quantities of 50 pmole. With some peptides, e.g. bacitracin, angiotensin II, quantities less than 25 pmole could still be visualized. On the other hand, peptides, e.g. oxytocin, which were not resolved as a distinct band, had a lower detection sensitivity than average. The reason for this difference is unclear. In general, however, the sensitivity of the present method is better than any of the fluorescence or micro gel electrophoresis procedures ever described. Besides the inherent advantages of using fluorescent labels this technique has the virtue of employing simple procedures and ordinary equipment for further improving the sensitivity. However, the major disadvantage of the present method is the difficulty of photographing the gels after electrophoresis.

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