ANALYTICAL BIOCHEMISTRY 67, 347–352 (1975)

Quantitation of Submicrogram Amounts of Protein Using Coomassie Brilliant Blue R on Sodium Dodecyl Sulfate–Polyacrylamide Slab-Gels

A sodium dodecyl sulfate (SDS)–polyacrylamide slab-gel system was used to study the use of Coomassie brilliant blue (CB) as a quantitative stain. Quantitation curves are shown for tubulin, cytochrome *c*, histone, and actin from gels stained with CB. A comparison was made of three identical gels using an actin sample and stained with CB, fast green and buffalo black. CB staining was found to be quantitative in the 0.05–2.75-μg range as well as possessing an order of magnitude increase in sensitivity over other stains tested.

Much of the recent work in quantitative polyacrylamide-gel electrophoresis (PAGE) is concerned with the stains and staining procedures. Some of the different stains used are fast green (1), amido black (2), and Coomassie brilliant blue (CB) (3). In most cases only a small percentage of the dye ever binds to protein. This prompted Datyner and Finnimore (4) to develop a method in which stain and protein are mixed under conditions favorable to the formation of covalent bonds before electrophoresis. They report a sensitivity down to 0.2 μg with some proteins. Another method, employed by Watkin and Miller (5), attempts to avoid the problems inherent in the use of dyes and staining by scanning gels immediately after electrophoresis under uv light at 280 nm. However, as is aptly pointed out (5), the problem of variable affinity for stain is exchanged for one of different relative absorbancies of light at 280 nm.

While CB is held by most workers to be the most sensitive dye available, there has been some doubt as to its value as a quantitative dye. Fishbein (3) has shown that CB could be quantitated in the 1–22-μg range. The major complaint against the use of CB as a quantitatively staining dye is that it shows deviation from Beer's Law at lower protein concentrations than the other dyes.

In this study CB was shown to bind quantitatively to various proteins at submicrogram concentrations not previously reported. The importance of the appropriate slab-gel system is also emphasized.

MATERIALS AND METHODS

Gels. The polyacrylamide thin (16 × 12 × 0.08 cm) slab-gel system of Laemmli (6) and O'Farrell *et al.* (7) was used, giving gels with an

concentration of 0.1%. Gels were made up no more than 72 nor less than 12 hr before use and were run at 10 mA through the spacer gel and 15 mA once into the lower gel.

Staining. Coomassie brilliant blue R (Sigma Chemical, lot #23C-0950), fast green FCF (Sigma Chemical, lot #70C-0580), and buffalo black NBR (Allied Chemical, lot #70C-0580) were used at a concentration of 0.1% in 25% trichloroacetic acid (TCA). Destaining took 2–3 days employing changes of 8% acetic acid. Gels were considered completely destained when there was no visible background stain remaining in the gel.

Samples. All proteins were made up in a sample buffer containing 10% glycerol, 5% 2-mercaptoethanol, 2% SDS, 8 M urea, 0.0625 M Tris, pH 6.8 with HCl. Histone from calf thymus (Sigma Chemical, lot #60C-2650) was prepared as a 1 mg/ml solution in sample buffer. Other samples included cytochrome *c* (horse heart, Sigma Chemical, lot #60C-2650) made up to 0.02 mg/ml, tubulin purified by vinblastine precipitation at a concentration of 0.012 mg/ml, and rabbit muscle actin, diluted to a final concentration of 0.025 mg/ml.

Quantitation. The stained bands were cut out of the slab gels using a razor blade and placed along the inside wall of a Gilford 2412 rectangular cuvette to which they adhered. Fresh, filtered 8% acetic acid was used to fill the cuvette in all work reported. The gels were scanned at 590 nm (red) with the use of a Gilford 240 spectrophotometer with a grid slit plate 2.36 × 0.05 mm and a slit width of 0.36 mm. Graphs were made by using a Gilson linear transport apparatus to move the gels past the spectrophotometer beam at the slowest scan speed (0.5 cm/min) and recorded on a Texas Instruments chart recorder at a speed of 7.6 cm/min.

Each gel had spaces for a maximum of 13 samples. No attempt was made to compare results from different gels. Four readings were taken of each gel as follows: The gel was read, flipped over and read again, a thin slice was taken off lengthwise and the gel was read again, and a second lengthwise slice was taken off for the fourth reading. This gave four distinct points on the protein band which were read and quantitated. The number of readings was later reduced to two per sample. The means of the areas under the peaks, determined by weighing on a Mettler balance, of the readings for each sample were then taken as a quantitative value.

RESULTS AND DISCUSSION

Electrophoresis of either pure proteins or whole tissue homogenates using cylindrical gels proved to be of insufficient sensitivity, particularly

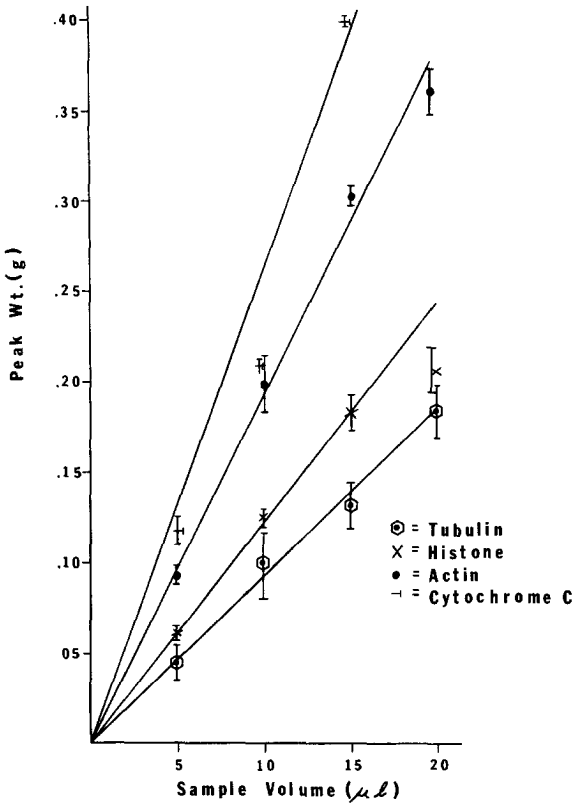

FIG. 1. Quantitation of four different proteins (tubulin, 0.012 μg/μl; actin, 0.025 μg/μl; cytochrome *c*, 0.02 μg/μl, and histone) at four sample volumes. Means and ranges are shown for each point.

gel due to the greater volume of the gel matrix. However, the greatest problem in using cylindrical gels was found to be in the staining. It was found that the stain does not penetrate the cylindrical gels uniformly or completely and thus could not be used for quantitative purposes. Penetration of the dye throughout the gel can be checked by slicing the gel with a razor blade in the middle of a peak and looking to see if the stain has penetrated to the center. When the penetration is incomplete there is a ring of stain with a clear center. Staining procedures were varied in efforts to get complete staining, but this was never achieved with cylindrical gels even after 2 days of staining or with the use of acetic acid, methanol, or trichloroacetic acid solutions. However, it was discovered that staining for 30 min. in a 0.1% CB solution in 25% TCA was sufficient for complete, uniform staining of a slab gel. When destained in

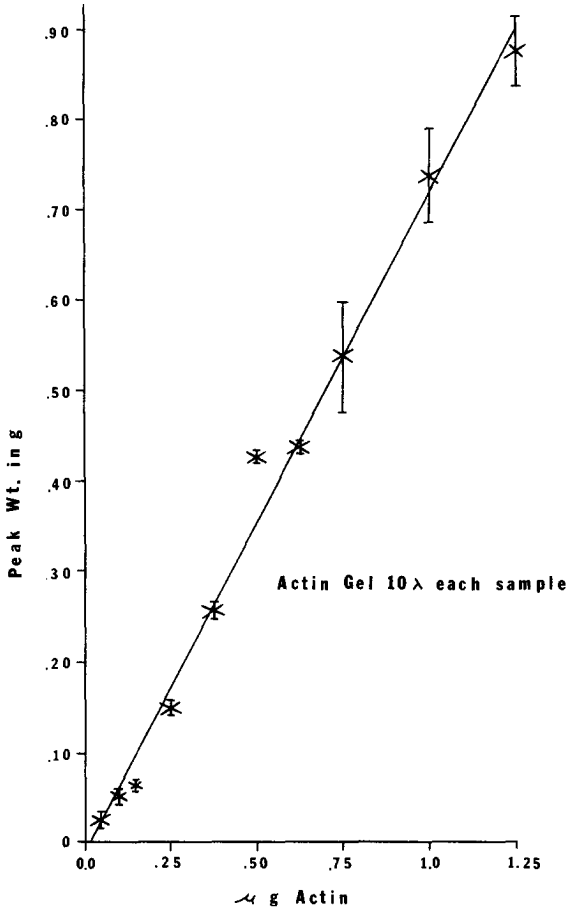

FIG. 2. Ten actin samples were made up with different protein concentrations. In each case the same sample volume (10 μl) is used to give concentrations from 0.05–1.25 μg. This graph represents one gel.

A variety of proteins were chosen to reflect differences in amino acid composition, net charge, and molecular weight. The data are shown (Fig. 1) for a histone protein (R_f, 0.591), tubulin, actin, and cytochrome *c*. It is noted that in most of these graphs at the greatest sample volume (20 μl) the value is slightly higher than that predicted from the line drawn through the other points. This was thought to be due to the increase in sample volume rather than a real deviation resulting from staining. This is supported by results shown in Fig. 2 in which ten different actin samples were prepared in different concentrations so that, when put on the gel, the same sample volume of 10 μl was used but different amounts

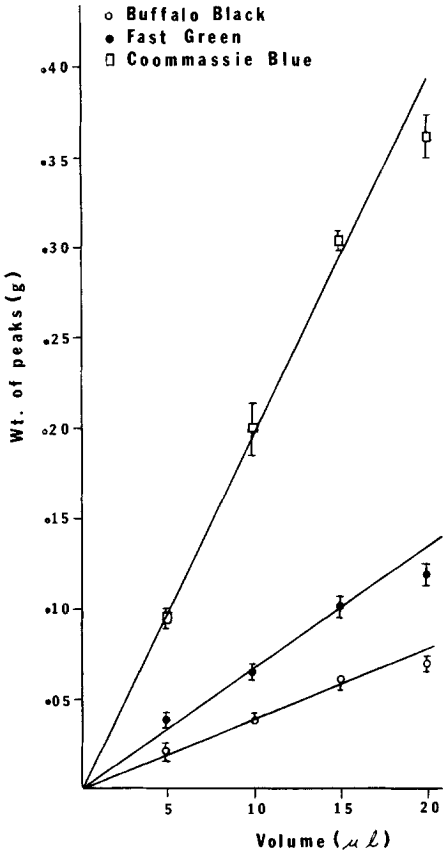

FIG. 3. Results of quantitation of three actin gels, identical in every respect, except for the stain employed.

Figure 3 illustrates the sensitivities of CB, fast green and buffalo black. At the same protein concentration the values from the CB-stained gel are almost an order of magnitude greater than peaks from gels stained with either fast green or buffalo black. The lowest amount of protein quantitated was 0.05 μg and could easily be seen by the eye. The greatest amount quantitated was 2.75 μg per band. We were thus able to quantitate protein bands with almost a hundred-fold range of sensitivity. Fishbein reports a range of 1–55 μg which probably reflects differences in the sensitivity of the spectrophotometer used in quantitating. It should be noted that with different instrumentation it should be possible to shift the range of concentrations which are quantitatable with the use of CB.

The utility of such a technique is perhaps best found in cellular and molecular research rather than as a strictly analytical method such as the

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.