Detection of Fluorescence Dye-Labeled Proteins in 2-D Gels Using an Arthur™ 1442 Multiwavelength Fluoroimager

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BioTechniques 31:146-149 (July 2001)

ABSTRACT

Labeling of proteins with SYPRO® Orange, SYPRO Red, and SYPRO Ruby after 2-D polyacrylamide gel electrophoresis (PAGE) using plastic-backed immobilized pH gradient (IPG) strips and pre-cast SDS polyacrylamide gels was tested. Protein spots were detected using an Arthur™ 1442 Multiwavelength Fluoroimager. The labeling methods described allow detection of proteins both after isoelectric focusing (IEF) and PAGE with a sensitivity higher than or comparable to standard silver staining methods. In addition to the post-labeling methods mentioned above, pre-labeling with the cysteine-specific fluorophore monobromobimane before 2-D PAGE is a sensitive, fast, and cost-effective alternative to existing staining protocols.

INTRODUCTION

High-resolution 2-D polyacrylamide gel electrophoresis (2-D PAGE) is a potent tool for the investigation of protein profiles from eukaryotic cells and bacteria. This technique separates proteins on the basis of their charge and their apparent molecular mass. Because of its high resolution, up to 5000–10 000 proteins could be visualized in a given sample if staining and detection of the protein spots are sensitive enough. While staining of proteins with Coomassie® Brilliant Blue dyes (R or G) has a rather limited sensitivity, silver staining methods are sensitive but are limited by their poor dynamic range, wide variability in response from protein to protein, procedural complications, and a subjective end point (i.e., the staining process has to be terminated at an individual time by the user). In contrast, detection methods using radio-labels for biosynthetic labeling are sensitive and can yield a good dynamic range if analyzed by a phosphorimager or mul-

ti-ple film exposures. However, they are inconvenient in practice because of handling safety considerations. In addition, not all samples are alive; therefore, serum and cryo-preserved biopsies cannot be labeled with isotopes. If biosynthetic labeling is possible (e.g., in a live biopsy), the protein expression pattern may change because of the non-native environment of the tissue (1).

Because of the development of suitable new and improved fluorophores and highly sensitive detection instruments, a growing number of fluorescence dye-based protein staining methods are now available. These methods can exhibit a sensitivity better than silver staining and a wider quantitative dynamic range (greater than 10³; for comparison of protein detection methods, see References 2, 3, 6, and 7, Table 1, and technical bulletins of various suppliers). Moreover, fluorescent dyes are easy and safe to handle, suitable for high-throughput approaches, and compatible with protein identification methods such as western blotting and mass spectroscopy.

Fluorescence dye staining was described previously (6,7,9) for 2-D PAGE using isoelectric focusing (IEF) gels in glass tubes and vertical slab gels in glass cassettes for SDS-PAGE. Recently, easy-to-handle precast immobilized pH gradient (IPG) strips became available and are now used in many laboratories, often with precast SDS polyacrylamide gels. These precast gels are plastic-supported, which often causes difficulties that are either due to the interaction of the fluorophore with the plastic backing or to the intrinsic fluorescence of the material. Here, we show that fluorescence dye staining can also be applied when using plastic-backed IPG strips and gels if a suitable filter combination and an Arthur™ 1442 Multiwavelength Fluoroimager (PerkinElmer Life Sciences/Wallac Oy, Cambridge, UK) is used for analysis.

Table 1. Comparison of Protein Detection Methods

Method	Technology	Sensitivity (ng protein/ 1-D band)	Dynamic Range	Limitations
Coomassie Blue	Absorbance	50	10 ²	Sensitivity, dynamic range
Silver	Absorbance	1	7	Dynamic range, complex staining procedure, subjective end point
Radioisotopes	X-ray film exposure	<1	10 ³ (multiple films)	Living samples Handling radioactivity
Radioisotopes	Phosphorimaging	<1	10 ⁴ –10 ⁵	Living samples Handling radioactivity, instrument availability
Fluorophores	Fluorescence	<1	10 ⁴ –10 ⁵	Instrument availability

Table 2. Filter Combinations for the Dyes Used in This Study

Dye	Excitation/ Bandwidth (nm)	Emission/ Bandwidth (nm)	Illumination	Exposure time (s)
Silver	-	530/30	bottom trans-illumination (using UV-to-white light converter)	0.05
MBB	390/70	480/30	edge	30–60
SYPRO Orange	480/30	590/30	top	10–30
SYPRO Red	540/30	620/30	top	10–30
SYPRO Ruby	480 or UV	620/30	top, UV trans-illumination	0.2–0.4

Figure 1. Comparison of MBB, silver, and SYPRO dyes staining results. Thirty-five micrograms of the soluble protein fraction were separated on the basis of their charge and their apparent molecular mass. The protein was either stained before 2-D PAGE using MBB (A) or after 2-D PAGE by silver (B), SYPRO Orange (C), SYPRO Red (D), and SYPRO Ruby (E) staining. Similar sections of the gels are shown. For MBB labeling, 8 μ L MBB (Thiolyte) stock solution in acetonitrile were added to 100 μ g protein in 350 μ L re-hydration solution and incubated for 25 min at room temperature in the dark. Subsequently 7 μ L 10% DTT solution were added, and an aliquot of 128 μ L corresponding to 35 μ g protein was used for 2-D PAGE.

MATERIALS AND METHODS

Sample Preparation

Cells of *Corynebacterium glutamicum* type strain ATCC 13032 were grown, and cell extracts were prepared by French Press treatment as described previously (4,5).

Labeling with Monobromobimane

Samples resuspended in sample buffer [4% CHAPS (Fluka, Buchs, Switzerland), 8 M urea, 0.5% Pharmalyte, 0.2% dithiothreitol (DTT) (Amersham Pharmacia Biotech, Freiburg, Germany)] were incubated for 15 min at room temperature with monobromobimane (MBB) (Thiolyte; Calbiochem-Novabiochem, San Diego, CA, USA; stock solution 200 mM in acetonitrile; final concentration 1.14 mM).

2-D PAGE and Image Analysis

IEF and SDS-PAGE were carried out as described (4,5) using either IPG strips and precast plastic-backed gels supplied by Amersham Pharmacia Biotech or Bio-Rad Laboratories (Munich, Germany). Labeling of proteins with SYPRO® dyes were carried out as described by the supplier (Molecular Probes, Leiden, The Netherlands), and silver staining was carried out according to Blum and co-workers (3). The image acquisition was carried out using an Arthur 1442 Multiwavelength Fluorimager. Filter combinations and illumination sources suitable for the different staining methods are described in Table 2 (extensive further information and consecutively improved protocols can be obtained from the corresponding author).

RESULTS

Labeling with MBB

MBB reacts rapidly and effectively with sulphhydryl groups of the protein cysteinyl residues. The derivative bound to the protein is highly fluorescent, while unreacted MBB is essentially non-fluorescent at the wavelength used. Since the MBB derivative is uncharged, it will not affect the proteins isoelectric point below pH 7.5, which is about one pH unit below the pK of the cysteinyl residues sulphhydryl group (pK = 8.5). MBB therefore effectively mimics the charge of the cysteinyl residues below pH 7.5. Although MBB is of small mass MBB (M_r 271.10), even less mass (i.e., only 190.91 Da) is added to a protein molecule per MBB derivative-conjugated cysteinyl residue because, during the nucleophilic substitution type reaction, the bromide and one hydrogen are leaving the molecule (9). In contrast to conventional staining with Coomassie Brilliant Blue or silver nitrate, proteins were pre-labeled before 2-D PAGE. Therefore, proteins were detectable not only after SDS-PAGE but also after IEF, and a quick control of IEF or SDS-PAGE using a standard UV trans-illuminator ($\lambda = 366$ nm) is possible. Depending on the labeling protocol used, the sensitivity of this method is less than silver staining (Figure 1, A and B). When bovine serum albumin, trypsinogen, and α -lactalbumin were labeled with MBB and proteins were separated on 1-D gels, the sensitivity was about 1 ng/band (P. Jackson, unpublished results).

Labeling with SYPRO Dyes

SYPRO Orange and SYPRO Red stains are designed for use with SDS gels and bind to the SDS associated with the protein molecules, while SYPRO Ruby can also be used to stain gels not containing SDS [e.g., non-denaturing gels because this dye is binding to positively charged lysyl, arginyl, and histidyl residues and to the amino terminal group of the protein (2)]. There was little difference found between SYPRO Orange and SYPRO Red stained gels, while SYPRO Ruby showed the highest sensitivity (Figure 1, C, D, and E) compared to silver staining (Figure 1B). However, MBB has a similar sensitivity to SYPRO Orange and SYPRO Red. Using proteins of the membrane fraction, which are difficult to stain because of their hydrophobicity (e.g., using Coomassie Brilliant Blue), similar results were obtained (data not shown).

DISCUSSION

In this study, we showed that using an Arthur 1442 Multi-wavelength Fluoroimager and fluorescence labeling of proteins separated on plastic-backed IPG strips or SDS gels is a fast, sensitive, and reliable method. Precast materials supplied by leading distributors such as Amersham Pharmacia Biotech, Bio-Rad Laboratories, and Genomic Solutions can be used. The tested fluorescent dyes applied as described here have different sensitivities; only the sensitivity of SYPRO Ruby was as good as the silver staining method used.

Both pre-labeling methods such as MBB labeling and post-labeling methods such as silver, Coomassie Brilliant Blue, and SYPRO dye staining have been investigated. Because the MBB derivative is uncharged and because of its small mass added to the cysteines of the proteins, the observed protein spot pattern is only marginally affected (Figure 1A) and can be directly compared with post-label staining (Figure 1, B–F). The pre-labeling dye MBB has the advantage of being cost effective and quick to use because gel processing and staining are no longer required. If protein samples are pre-labeled with a set of reactive dyes (i.e., a set of mass and charged matched fluorophores) that react with same specificity but that have different spectral properties, the labeled samples can be mixed and run simultaneously in the same gel. Then, a direct comparison of protein spots will be possible without gel matching (8).

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