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A comparative study of different dyes for the detection of proteomes derived from *Escherichia coli* and MDCK cells: Sensitivity and selectivity

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ABSTRACT

We compared sensitivity and selectivity of five dyes for detection of 2D PAGE-resolved proteins derived from *Escherichia coli* and MDCK cells. The sensitivity of these dyes was in the following order: SYPRO Ruby > Deep Purple > CBB-G250 > CBB-R250 > Colloidal Gold. Also, we report herein for the first time the application of Colloidal Gold (which is commonly used for staining proteins on blotted membranes) for in-gel staining of proteins. For *E. coli*, most of the dyes preferably detected proteins with pI range of 4.0–6.9, whereas Deep Purple preferably detected proteins with less acidic range (pI 5.0–7.9). For MDCK cells, while other dyes preferably stained proteins at pI range of 5.0–7.9, Colloidal Gold preferably stained more basic proteins (pI 7.0–9.9). This preferential staining property of Colloidal Gold to basic proteins was confirmed in SDS-PAGE-separated lysozyme (pI 9.4), compared to calmodulin (pI 4.0) and albumin (pI 6.0). These data provide useful information to select appropriate dyes for gel-based proteomic analysis of individual samples.

1. Introduction

Two-dimensional polyacrylamide gel electrophoresis (2D PAGE) remains the most commonly used method in proteomic analysis. Common dyes, which are used for detecting the resolved protein spots in most of proteomic laboratories, include Coomassie Brilliant Blue R250 (CBB-R250), CBB-G250, silver and fluorescent stains. CBB-R250 is probably the most popular dye for protein detection because of its ease of use, affordable cost, and compatibility with subsequent analysis by mass spectrometry (MS). CBB is a sulfonated triphenylmethane dye, which has a positively charged quaternary nitrogen–carbon group in its anionic species, two negatively charged sulfonic groups, and one proton-neutralized sulfonic group in its neutral species. The neutral-species molecules will bind to protein molecules using hydrophobic interactions [1,2]. However, its sensitivity to detect protein spots resolved in 2D gels is considerably low. CBB-G250 had been later developed [3] to increase the sensitivity of CBB staining by salting-out ions such as large amount of ammonium sulfate, which enhances hydrophobic interaction [4]. By this modification, CBB-G250 can detect proteins at a smaller amount (as low as nanogram level) with a linear-dynamic response of spot/band intensities in association with the protein amounts [5].

The most sensitive dye available in almost all of basic, molecular, and proteomic laboratories is silver stain. Although it offers the highest sensitivity (down to low nanogram level [6,7]) its use in a proteomic study is limited because of its narrow linear-dynamic range [8] and difficulty of subsequent MS analysis, particularly when glutaraldehyde is used during color developing step [9]. Glutaraldehyde can irreversibly cross-link with polypeptide chains resulting to mass shift and can also prohibit proteolytic digestion. Subsequently, formaldehyde has been employed to replace glutaraldehyde and several studies have shown the compatibility of this modified silver staining for MS analysis [10]. However, formaldehyde can form a Schiff base with free amino groups causing poor tryptic digestion [11]. Even with this improved MS compatibility, its narrow linear-dynamic range still limits its use in comparative or differential proteomics studies [8].

Fluorescent dyes (i.e., SYPRO Ruby and Deep Purple) had been introduced to the proteomics arena to overcome the limitations of the aforementioned colorimetric stains. They have comparable sensitivities as of silver stain, whereas their linear-dynamic ranges are as broad as CBB stain [12]. SYPRO Ruby is a bathrophenanthroline complex of ruthenium (II) that is associated with cationic

Abbreviations: 2D PAGE, two-dimensional polyacrylamide gel electrophoresis; CBB, Coomassie Brilliant Blue; CHAPS, 3-[(3-cholamidopropyl) dimethyl-ammonio]-1-propanesulfonate; dI, deionized; DIGE, difference in-gel electrophoresis; DTT, dithiothreitol; EDTA, N,N,N′,N′-ethylenediaminetetraacetic acid; IEF, isoelectric focusing; MDCK, Madin–Darby canine kidney; MS, mass spectrometry; pI, isoelectric point; SDS, sodium dodecyl sulphate.

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residues on proteins [13]. Its staining mechanism is primarily through electrostatic interaction, and also possibly through coordination of the ruthenium atom with N and O of the polypeptide backbone [5]. Deep Purple is a novel fluorescent natural product derived from the fungus *Epicoccum nigrum*. Former studies have shown that it is very sensitive and can detect proteins in the picogram range [14,15]. When the epicocconone (a non-fluorescent azophilone) reacts with basic amino acids, primary amine and NH_3, and forms an internal charge transfer complex, this complex is highly fluorescent in the orange or red ranges [14,16–19]. Moreover, proteins covalently bound to the epicocconone are reversible, depending on their pH [19]. Thus, it is easily removed prior to tryptic digestion and compatible with MS analysis.

In addition to the aforementioned dyes, Colloidal Gold particle has been found applicable in biomedical research [20–22]. This particle has been applied to visualize proteins on membranes or blots in many reports [23,24]. Colloidal Gold-stained blots have been found to be more efficient and have higher sensitivity than other reversible stains [25]. Under acidic condition, Colloidal Gold particle containing thousands of atoms can be attained by oxidative gold metal dissolution to limited detection at picomolar level [26]. However, this blot stain has never been applied to visualize proteins resolved in 2D gels; thus, its applicability and sensitivity in comparison to other dyes remain unknown.

Because of different mechanisms of staining by these dyes and their differential advantages/disadvantages, selection for the appropriate dye for a gel-based proteomics study is quite crucial to determine the quantitative and qualitative changes in the targeted proteomes [18,27]. Indeed, there are a number of previous studies, which compared the applicability of a few of these dyes in-gel-based proteomics [5,8,13,28,29]. However, none of these studies have evaluated the selectivity of particular components of the proteome detectable by different dyes (as almost all of them evaluated mainly on the sensitivity, range of detection, and MS compatibility). Additionally, Colloidal Gold (blot stain) has not been previously evaluated for its applicability to stain proteins in-gel.

Our present study, therefore, evaluated both sensitivity and selectivity of five different dyes, including Colloidal Gold, CBB-R250, CBB-G250, Deep Purple, and SYPRO Ruby for their detectability of proteins resolved in 2D gels. Silver staining, which is well known as the most sensitive stain, was not included into this study because of its limitation in quantitative proteomics study using 2D PAGE [8]. The sensitivity was determined by total number of protein spots and integrated intensity levels of all these spots detected in each gel, whereas the selectivity was determined by the detectability of proteins within each isoelectric point (pI) or molecular weight (MW) range. In the present study, we used only pI and MW ranges for evaluation of the selectivity because we wished to examine staining properties of these dyes on proteins with differential pH and molecular masses first. Indeed, there are several other physicochemical properties of proteins that can be evaluated further for the selectivity. We also evaluated whether the sensitivity and selectivity were dependent on sample types by comparing the detectability of these dyes on proteins derived from two different samples: *Escherichia coli* (as a representative bacterial sample) and Madin–Darby canine kidney (MDCK) cells (as a representative mammalian sample). We used these two cell types because our initial data showed that the 2D proteome profiles of these two samples obviously differed. We did not use a mixture of known proteins because the data will be limited only to the known proteins and not applicable to the wide variety of proteins presented in the whole cellular proteomes, which have much more variations in pH and molecular masses.

2. Materials and methods

2.1. Sample preparation

2.1.1. E. coli cell extract

E. coli DH5-α strain was grown in 10 mL Luria–Bertani broth containing 1.0% tryptone, 0.5% yeast extract and 1.0% sodium chloride at 35 °C overnight under aerobic condition. The *E. coli* pellet was then collected by centrifugation at 10,000 × *g* for 5 min, washed twice with phosphate buffered saline (PBS), and resuspended in a lysis buffer containing 7 M urea, 2 M thiourea, 4% 3-[(3-cholamidopropyl) dimethyl-ammonio]-1-propanesulfonate (CHAPS), 2% ampholytes pH 3–10, 120 mM dithiothreitol (DTT) and 40 mM Tris–HCl at 4 °C for 30 min. Unsolubilized debris was removed by centrifugation at 20,000 × *g* for 10 min and the protein concentration was then measured by the Bradford method.

2.1.2. MDCK cell extract

MDCK cells were inoculated in 75-cm^2 tissue culture flask containing a complete Eagle's minimum essential medium (GIBCO, Invitrogen Corporation; Grand Island, NY) supplemented with 10% fetal bovine serum, 1.2% penicillinG/streptomycin, and 2 mM glutamine. The cultured cells were maintained in a humidified incubator at 37 °C with 5% CO_2 for 48 h. The monolayer of MDCK cells was harvested by trypsinization (0.1% trypsin in 2.5 mM EDTA) for 2–5 min at 37 °C, and then centrifugation at 10,000 × *g* for 2 min. The cell pellet was washed with PBS three times and solubilized with the lysis buffer as aforementioned. Unsolubilized debris and particulate matters were removed by centrifugation at 10,000 × *g* for 2 min and the protein concentration was then measured by the Bradford method.

2.2. Two-dimensional polyacrylamide gel electrophoresis (2D PAGE)

2D PAGE was run in triplicate for both *E. coli* and MDCK samples, and for all stains. Samples (equally 100 μg total protein for each sample) were mixed with a rehydration buffer (7 M urea, 2 M thiourea, 2% CHAPS, 120 mM DTT, 40 mM Tris-base, 2% ampholytes (pH 3–10), and a trace of bromophenol blue) to a final volume of 150 μL per sample. The mixtures were then rehydrated onto Immobiline DryStrip (linear pH gradient of 3.0–10.0, 7 cm long) (GE Healthcare; Uppsala, Sweden) at room temperature for 15 h. The first dimensional separation or isoelectric focusing (IEF) was performed in Ettan IPGphor II IEF System (GE Healthcare) at 20 °C, using a stepwise mode to reach 9083 V h. After completion of the IEF, the strips were first equilibrated for 15 min in an equilibration buffer (6 M urea, 130 mM DTT, 112 mM Tris-base, 4% sodium dodecyl sulphate (SDS), 30% glycerol, and 0.002% bromophenol blue) and then in another similar buffer, where DTT was replaced with 135 mM iodoacetamide, for 15 min. The second dimensional separation was performed in 12% polyacrylamide gel using SE260 mini-Vertical Electrophoresis Unit (GE Healthcare) at 150 V for approximately 2 h.

2.3. 1D PAGE (SDS-PAGE)

Lysozyme (Sigma–Aldrich; St. Louis, MO) (with a pI of 9.4; as a representative basic protein); calmodulin (Sigma–Aldrich) (with a pI of 4.0; as a representative acidic protein); and albumin (Sigma–Aldrich) (with a pI of 6.0; as an intermediate protein with a pI between lysozyme and calmodulin) were equally loaded with an identical 2-fold dilution series of their total amounts (up to 10 μg). 1D PAGE (SDS-PAGE) was performed in 12% polyacrylamide gel using SE260 mini-Vertical Electrophoresis Unit (GE Healthcare) at 150 V for approximately 2 h.