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Improved Ruthenium II tris (bathophenantroline disulfonate) staining and destaining protocol for a better signal-to-background ratio and improved baseline resolution

In proteomics the ability to visualize proteins from electropherograms is essential. Here a new protocol for staining and destaining gels treated with Ruthenium II tris (bathophenantroline disulfonate) is presented. The method is compared with the silver-staining procedure of Swain and Ross, the Ruthenium II tris (bathophenantroline disulfonate) stain described by Rabilloud (Rabilloud T., Strub, S. M. Luche, S., Girardet, S. L. *et al.*, *Proteomics* 2001, *1*, 699–704) and the SYPRO Ruby gel stain. The method offers a better signal-to-background ratio with improved baseline resolution for both sodium dodecyl sulfate-polyacrylamide gels and two-dimensional gels.

Keywords: Baseline resolution / Destaining / Ruthenium II tris (bathophenantroline disulfonate) / Signal-to-background ratio PRO 0587

1 Introduction

There exist different staining methods to visualize proteins in polyacrylamide gels. The most sensitive is autoradiography of proteins radiolabelled biosynthetically or by radio-iodination. This method is unsurpassed for analytical purposes. It is inconvenient if proteins have to be isolated because the correlation between signals on a film and the invisible protein spot in the gel is difficult without special equipment. Silver-staining is the most efficient method for direct in-gel visualization. The dynamic range of this method however, is limited due to saturation effects. Staining is time-dependent, not an equilibrium reaction, and color as well as intensity of staining vary with type of protein in an idiosyncratic way [1]. Silver-stained proteins are difficult to analyze by MS. Staining with Coomassie Blue and Colloidal Coomassie Blue does not have any of these drawbacks but is less sensitive.

It has long been known that proteins covalently labelled with fluorescent dyes can be detected with high sensitivity. More recently noncovalent fluorescent stains have been introduced such as SYPRO Ruby, other dyes of the SYPRO family and ruthenium II tris-bathophenantroline disulfonate (RuBP) as described by Rabilloud *et al.* [2, 3]. While SYPRO Ruby is a ready to use formula, RuBP offers several possibilities for improvement and is cheaper. A number of modified staining protocols have been published for RuBP [4, 5] and SYPRO Ruby [4–7], and destaining protocols for iron II tris (bathophenantroline disulfonate) [8], SYPRO Ruby (Voshol, H, Novartis, private communication) [5] and europium tris (bathophenantroline disulfonate) [9]. The RuBP staining procedure by Rabilloud *et al.* was improved mainly by optimizing reagent concentration, pH, and solvent composition for both the staining and destaining steps. Here it is compared with silver-staining according to Swain and Ross (modified) [10], SYPRO Ruby staining according to Berggren *et al.* [4] and ruthenium tris (bathophenanthroline disulfonate) staining according to Rabilloud *et al.* [2].

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Abbreviations: AIDA, abvanced image data analyzer; **LAU**, linear arbitrary units; **RuBP**, ruthenium II tris-bathophenantroline disulfonate

2 Materials and methods

2.1 Synthesis of RuBP

RuBP was prepared as published by Rabilloud *et al.* [2] and used without further purification. The UV/visible spectrum of the product was identical to the published spectrum [3].

2.2 SYPRO® Ruby protein stain

SYPRO Ruby was purchased from Bio-Rad Laboratories (170–3125; Richmond, CA, USA). Check of the lot number confirmed the use of the new SYPRO Ruby formulation as described in [11].

2.3 PAGE

Marker proteins (17–0446–01; Amersham Biosciences, Uppsala, Sweden) were serially diluted 1:2 (phosphorylase b 3350 ng to 0.82 ng, albumin 4150 ng to 1 ng, ovalbumin 7350 ng to 1.8 ng, carbonic anhydrase 4150 ng to 1 ng, trypsin inhibitor 4000 ng to 1 ng, lactalbumin 5800 ng to 1.4 ng) and separated on a 17.5% gel prepared as described [12] in a Protean 3 Mini cell system (Bio-Rad).

2.4 2-DE

(i) Analytical gels: For the first dimension, IPG strips (pH 3–10; Amersham Biosciences) were rehydrated for 10 h with 300 μL sample buffer (8 M urea, 2% CHAPS, 18 mM dithioerythritol (DTE), 0.5% IPG buffer 3–10 and traces of Bromophenole Blue) containing 80 μg *Escherichia coli* proteins. IEF was carried out in an IPGphor (Amersham Biosciences) with the following settings: 20°C, 200 μAmp *per* strip, 150 Vh (1 h, 150 V, step-n-hold), 300 Vh (1 h, 300 V, step-n-hold) 17 500 Vh (5 h, 3500 V, step-n-hold) 63 250 Vh (gradient). (ii) Preparative gels: IPG strips were rehydrated with 300 μL sample buffer containing 800 μg *E. coli* proteins for 10 h on the IPGphor under 30 V. Focussing was carried out with the following settings: 20°C, 200 μm Amp *per* strip, 150 Vh (1 h, 150 V, step-n-hold), 300 Vh (1 h, 300 V, step-n-hold) 17 500 Vh (5 h, 3500 V, step-n-hold) 27 6000 Vh (gradient), 80 000 Vh (10 h, 8000 V, step-n-hold). Second dimension (for i and ii): The IPG strips were equilibrated for 12 min with 5 mL/strip of solution I (50 mM Tris-HCl, pH 7.0, 6 M urea, 30% v/v glycerol, 2.5% w/v DTE) and 12 min with 5 mL/strip of solution II (50 mM Tris-HCl, pH 7.0, 6 M urea, 30% v/v glycerol, 2.5% w/v iodoacetamide). Before loading on the second dimension 1.5 cm of the basic end from the IPG strip was cut off. The second dimension was run on a 12% polyacrylamide gel (15 × 16 cm^2) at 2°C for 5 h at 50 mAmp *per* gel (500 V) in a Hoefer Dalt 600 chamber (Hoefer Scientific Instruments, San Fransico, CA, USA).

2.5 Staining procedures

A volume of 200 mL in each step of staining or destainig was used. Analytical gels were stained as detailed in protocols 1–4 (see Tables 1–4) with (i) silver nitrate as described by Swain and Ross [10] (with modifications), (ii) with SYPRO Ruby as described by the manufacturer, (iii) with RuPB according to the procedure of Rabilloud *et al.* [2], and (iv) with RuPB as follows: Gels were incubated in 30% ethanol and 10% acetic acid for 15 h (overnight), washed four times for 30 min each with 20% ethanol, and then stained with 1 μM RuBP in ion-exchange water. Staining was done in a stainless steel tray, in the dark, on a shaker for six h. The gels were destained by rinsing with water two times for 10 min, and then incubated in 40% ethanol/10% acetic acid for 15 h or overnight. The destained gels can be stored in water for several days without loss of signal intensity and can be silver-stained for further analysis [13].

Table 1. Protocol 1: Silver-staining protocol (Swain and Ross [4] modified)

Step	Procedure	Comments
1	Fix the gel in 40% EtOH/10% acetic acid for 2 h	For the sake of convenience the gel can be fixed overnight
2	Incubate the gel in 40% EtOH, containing 10 μL of 37% formaldehyde for 5 min	
3	Wash with 40% EtOH for 20 min	
4	Rinse for 20 min with water	
5	Incubate the gel for 1 min in sodiumthiosulfate (20 mg/100 mL)	
6	Stain with 1 g silvernitrate *per* 1000 mL for 20 min	
7	Rinse for one min with water and repeat three times	Do not rinse longer than one min each time
8	Develop with a solution of 25 g sodiumcarbonate and 400 μL 37% formaldehyde in 1000 mL water for 4 min and 15 s	After 1 min and 15 s the first spots become visible. If the solution turns yellow, it must be removed and replaced with an equal amount. Note: the best is to use a system that can remove at least 50 mL solution *per* second. Work has to be done hand free on all stages!
9	Stop developing with 10% acetic acid containing 0.6% Tris base for 5 min	
10	Rinse the gel with water for 5 min	Scan the gel immediately after development. Color varies even after a short time
11	Store the gel in 1% acetic acid at 4°C	

Table 2. Protocol 2: SYPRO Ruby gel stain [5]

Step	Procedure	Comments
1	Fix the gel in 10% MeOH, 7% acetic acid for 30 min	
2	Incubate the gel in SYPRO Ruby staining solution for 3 h-overnight	
3	Wash the gel in 10% MeOH, 7% acetic acid for 30 min and scan	
	all % are in V/V	

Table 3. Protocol 3: Ruthenium II tris (bathophenantroline disulfonate) staining protocol according to T. Rabilloud *et al.* [2]

Step	Procedure	Comments
1	Fix the gel in 30% EtOH, 10% acetic acid overnight	
2	Rinse the gel in 20% EtOH for 30 min and repeat 3 times	
3	Incubate the gel in 100 nM RuBP solution for 6 h	The concentration of the stock solution is 20 mM. Dilute 5 μL in 1000 mL 20% EtOH just prior to use
4	Equilibrate the gel in water for 10 min, repeat once and scan	
	all % are in V/V	

Table 4. Protocol 4: Ruthenium II tris (bathophenantroline disulfonate) new staining/destaining protocol [13]

Step	Procedure	Comments
1	Fix the gel in 30% EtOH, 10% acetic acid overnight	
2	Rinse the gel in 20% EtOH for 30 min and repeat 3 times	
3	Incubate the gel in 1 μM RuBP solution for 6 h	The concentration of the stock solution is 20 mM. Dilute 50 μL in 1000 mL water just prior to use
4	Equilibrate the gel in water for 10 min and repeat once	A first scan is possible at this stage
5	Destain the gel with 40% EtOH/10% acetic acid for 15 h	
6	Equilibrate the gel in water for 10 min repeat once and scan	
	all % are in V/V	

2.6 Imaging

Silver-stained gels were scanned on a flatbed scanner (HP Deskscan, DeskScanII V2.3) with the following scanning parameters: 300×300 dots *per* inch, eight bit black and white picture (256 grey shades, two times sharpened), contrast 125, brightness 125. SYPRO Ruby and RuBP-stained gels were scanned with a Phosphorimager (Fuji FLA-3000 from Raytest with software BASReader V3.01 Straubenhardt, Germany) using the following scanning parameters: resolution 50 μm, 16 bit picture (65 536 grey shades), sensitivity 1000, excitation wavelength 473 nm and detection filter O580. Images were processed with advanced image data analyzer (AIDA) V3.10. Profiles were drawn with the 1-D evaluation tool of AIDA V3.11. 2-D Densitometric analysis was done with the 2-D densitometry module of AIDA 3.11.002. First the protein spots were captured. Then peak volumes

over background were calculated by numeric integration of the grey values of each pixel within a spot area. The background was measured in a rectangular box of 5 mm^2 near the spot and was subtracted from the numerical integral *per* area.

2.7 Protein identification

Coomassie or RuBP-stained protein spots were cut out of the gel. The gel pieces were destained with 100 mM ammonium bicarbonate in 30% ACN. Proteins were digested with trypsin [14] and peptide masses were identified by MALDI TOF as described [15]. The probability of a false positive match of an observed MS-spectrum was determined for each analysis [16].

3 Results and discussion

3.1 SDS PAGE

Two-fold serially diluted marker proteins were separated by SDS-PAGE (Fig. 1) and stained with silver, SYPRO Ruby and RuBP respectively, according to the protocols listed in Tables 1–4. Gels stained with RuBP and SYPRO Ruby were scanned in the phosphor-imager FLA-3000 (Raytest). Silver-stained gels were scanned on a flatbed scanner, and images were transformed to linear Arbitrary Units (LAU) with AIDA. Silver-staining produces uneven staining. Protein bands stained with fluorescent dyes have an overall balanced appearance. Peak volumes over background were used to measure the quantity of protein. A plot of signal intensities (integral/area-background) *versus* protein amount obtained from 2-D densitometric evaluation is shown for each protein and staining technique in Fig. 2. The threshold of detection was 2 ng for the silver-stain procedure (Lactalbumin, 14.1 kD), 4 ng for SYPRO Ruby (Trypsin inhibitor, 20.1 kD), 16 ng for the RuBP stain (Carbonic anhydrase, 30 kD) according to [2] and 8 ng for the modified RuPB procedure (Trypsin inhibitor, 20.1 kD). Silver-staining was the most sensitive method for all quantified proteins. SYPRO Ruby, which was second best in sensitivity, did not show as good linear dose/response behavior and had less intense signals than the modified RuBP stain which gave the strongest signals and best linear behavior (Fig. 2). Staining of 1-D gels with silvernitrate, according to the modified RuBP procedure, gave an artefact caused by the gel electrophoresis unit. It occured in the 60 kD region. This artefact shows up frequently in gels in our laboratory. It occurs in all types of analysis for all types of samples from different species in varying intensities. It can not be avoided. It may originate from traces of detergent used to cleanse the glass plates.

The behavior of signal-to-background was investigated by profiling and 2-D densitometry. Profile curves of lanes 1, 4 and 7 from each gel were recorded (Fig. 3) by scanning the lanes from edge to edge along a 1.05 mm wide rectangular field at their centre. Areas at the upper and lower edge of the lanes containing no protein were chosen to determine the background. The peak heights obtained with silver-staining are unrelated to the protein amount and therefore cannot be used for quantification. Peak heights obtained with fluoresencent dyes are proportional to the protein amounts but the relative intensities vary from scan to scan as is obvious from a comparison of the scans of lanes 1, 4 and 7. The signal-to-background ratio was 60/0.2 for the improved RuBP procedure, and 7/0.8 and 23/3 for SYPRO Ruby and

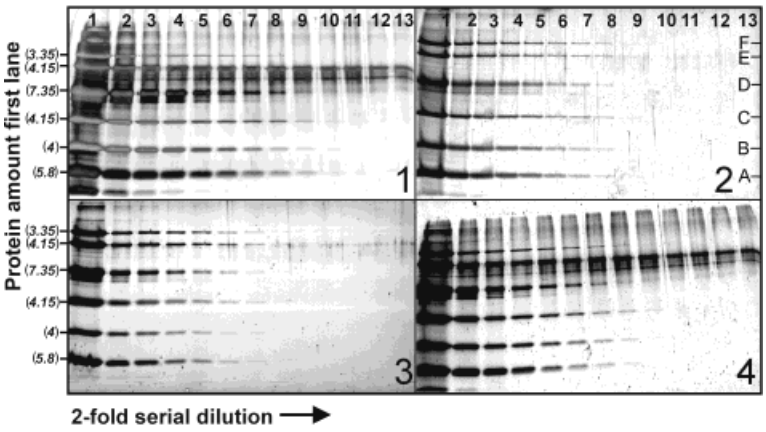

Figure 1. Signal strength and quality and background staining by different protein detection procedures. (1) Silver-stain; (2) SYPRO Ruby Protein Gel Stain; (3) Ruthenium bathophenantroline disulfonate according to the procedure of Rabilloud *et al.;* (4) modified Ruthenium bathophenantroline disulfonate procedure. Marker proteins were subjected to two-fold serial dilution. (A) Lactalbumin, 14.4 kD (5800 ng), (B) Trypsininhibitor, 20.1 kD (4000 ng), (C), Carboanhydrase, 30 kD (4150 ng), (D) Ovalbumin, 45 kD (7350 ng), (E) Albumin, 67 kD (4150 ng), (F) Phosphorylase b, 94 kD (3350 ng). The exact amount of each protein in lane 1 is indicated in parenthesis. Notice, that staining with silver (1) and RuBP (4) produces an electrophoresis artefact in the 60 kD region of the gel.

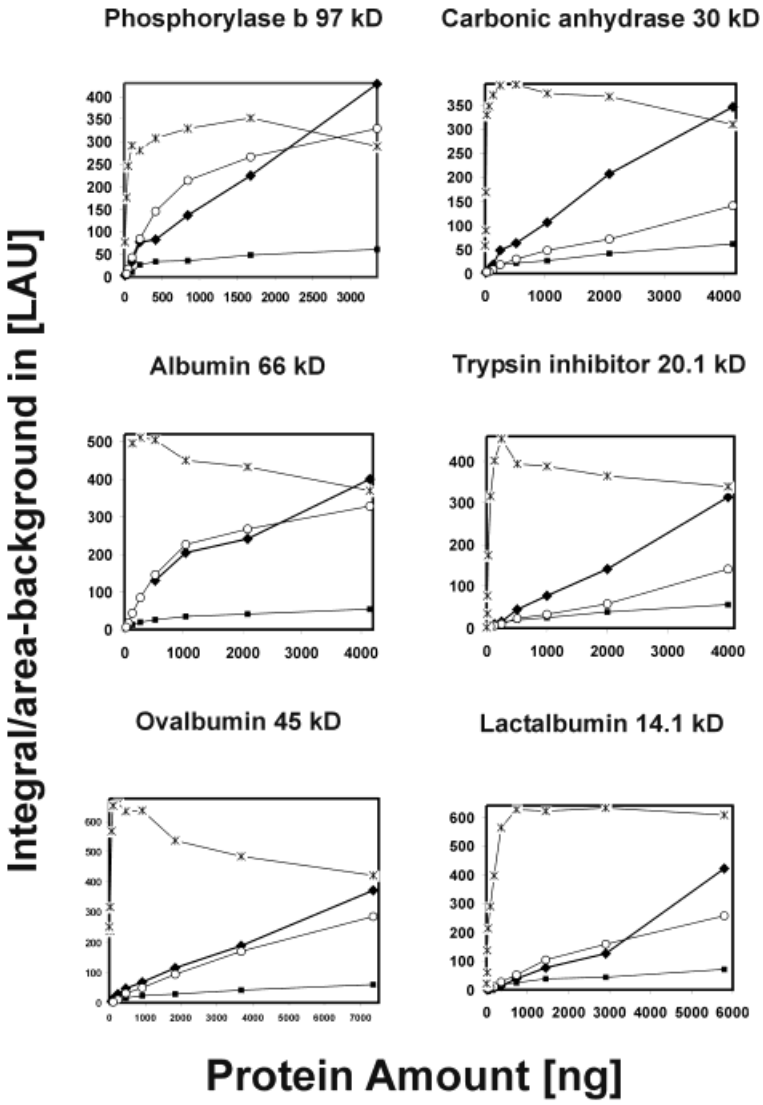

Figure 2. Plot of signal intensities (integral/area-background) *versus* protein amount obtained from 2-D densitometric evaluation. The symbols used are as follows: modified RuBP stain, diamonds; silver-stain, stars; SYPRO Ruby, squares; RuBP according to the procedure of Rabilloud *et al.,* open circles.

RuBP staining [2] respectively. Even at the highest protein amount/band, the fluorescent stains did not produce flat peaks (Fig. 3, lane 1). The artificial band mentioned above produces a peak of 4 LAU in all lanes of the gel stained with the new method. As it comigrates with albumin, both peaks of the profile overlap. In lanes 1 to 4 the albumin peak is dominant. In lane 7 the peak caused by the artefact is dominant over the albumin peak which is not visible.

Peak height can be affected by local anomalies and bands often are unevenly distributed across a lane. Peak height measurement is therefore potentially erroneous. To circumvent this, the peak volumes over background were compared to background using the 2-D densitometry Module of AIDA. Figures 4, 5, and 6 show plots of the ratio of signal intensities-to-background (integral/area-background) *versus* protein amount. The modified RuBP staining gave the best ratio with a maximum ratio of 74.2 for Lactalbumin, which was up to 12-fold stronger than the second best ratio (Fig. 4). The good linear behaviour becomes visible here. In Figure 5 the signal intensity scale is restricted to 10 LAU and below. SYPRO Ruby and RuBP staining according to the procedure of Rabilloud *et al.* produced signals up to 6.1 LAU. Silver-staining showed the typical curve of saturation. The plots of protein amounts below 700 ng for Lactalbumin, 450 ng for Ovalbumin and 250 ng for the other proteins tested are shown in Fig. 6. For protein loads below 50 ng silver-staining always produced the best signal-to-background ratios. The only exception was seen for Phosphorylase b where the modified RuBP procedure

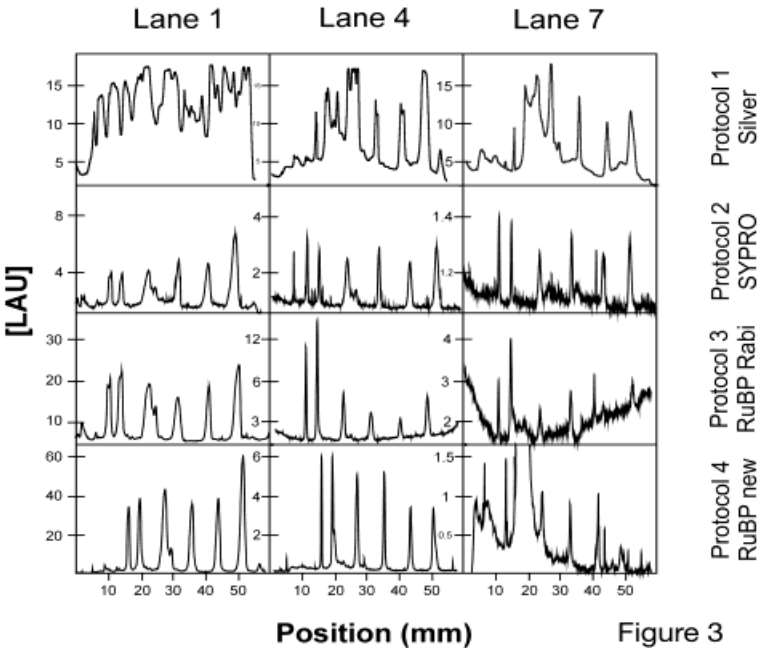

Figure 3

Figure 3. Profile scans of lanes 1, 4, and 7 from gels shown in Fig. 1. Different staining procedures produce markedly different signal intensities (y-axis). The signal-to-background ratio is close to 200:1 for the modified RuBP staining procedure, 5:1 for SYPRO Ruby, 8:1 for the RuBP procedure by Rabilloud *et al.,* and 6:1 for the silver-stain procedure.

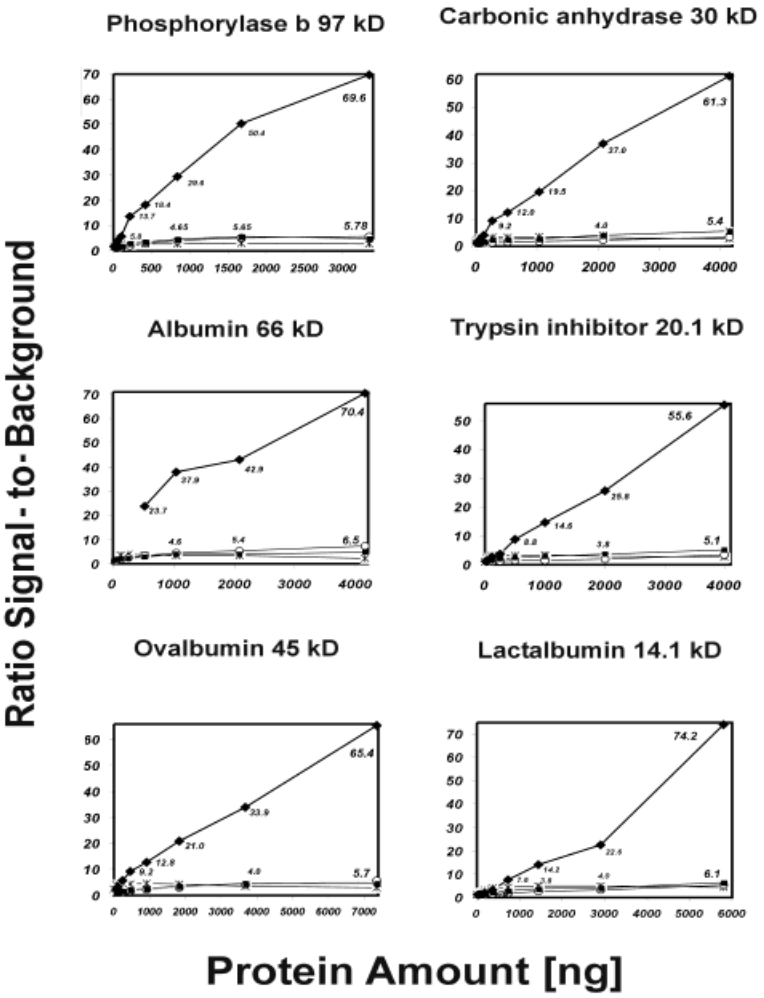

Figure 4. Plot of the ratio of signal intensities-to-background (integral/area-background) *versus* protein amount obtained from 2-D densitometric evaluation. The symbols used are as follows: modified RuBP stain, diamonds; silver-stain, stars; SYPRO Ruby, squares; RuBP according to the procedure of Rabilloud *et al.,* open circles. The signal-to-background values are indicated for the new RuBP stain and the second best stain. The Modified RuBP stain generated a maximum ratio of 74.2 compared to the second best maximum ratio of 6.5 from the other staining methods. The modified RuBP staining showed the best linearity.

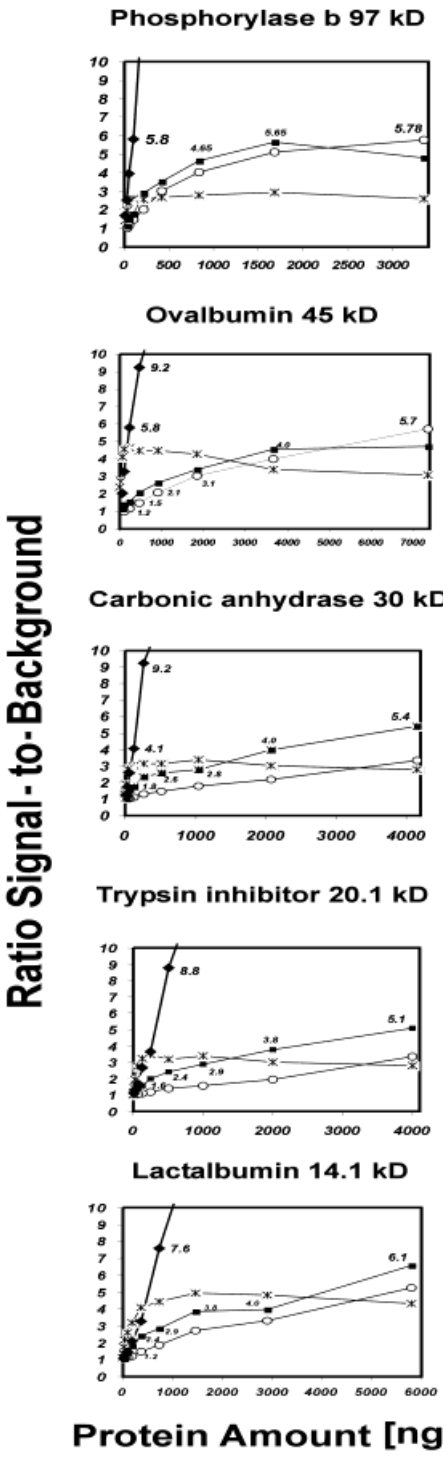

Figure 5. Plot of the ratio of signal intensities-to-background *versus* protein amount for ratios below 10. The symbols used are as follows: modified RuBP stain, diamonds; silver-stain, stars; SYPRO Ruby, squares; RuBP according to the procedure of Rabilloud *et al.,* open circles. Silver-staining reached saturation. The RuBP stain according to Rabilloud *et al.* and the SYPRO Ruby Protein stain showed a slow increase in ratio whereas the modified RuBP stain was out of scale.

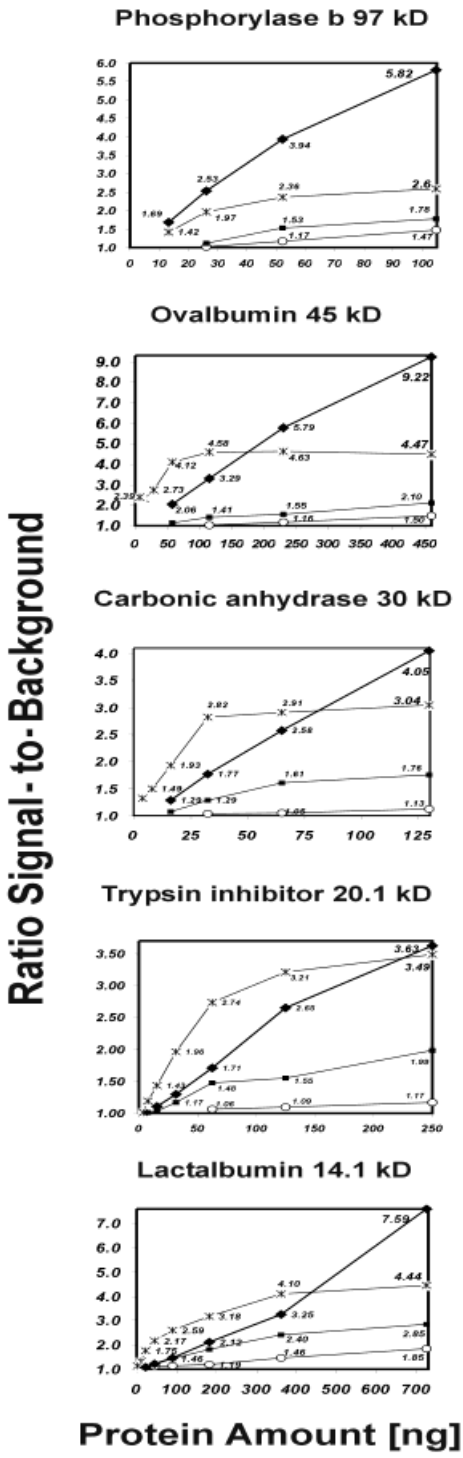

Figure 6. Plot of the ratio from signal intensities-to-background *versus* protein amount for protein quantities below 700 ng and ratios below 9. The symbols used are as follows: modified RuBP stain, diamonds; silver-stain, stars; SYPRO Ruby, squares; RuBP according to the procedure of Rabilloud *et al.,* open circles. For low protein amounts, silver-staining showed the best sensitivity but early onset of saturation whereas the modified RuBP staining has better linearity.

was better. For all other proteins, the second best values were obtained with the modified RuBP procedure. SYPRO Ruby can only compete for Lactalbumin at the two lowest concentrations.

Compared to SYPRO Ruby protein staining, the modified RuBP procedure gives better contrast but is less sensitive. This is due to the final destaining step. During destaining RuBP molecules are removed selectively from the gel matrix but not from proteins. For low protein concentrations the destaining time might be too long. Therefore, these proteins are destained too much and loose their fluorescence. Shorter destaining times may optimize the procedure resulting in greater sensitivity.

3.2 2-D gels

Protein expression proteomics requires the quantitative comparison of samples that differ by multiple small variables. The significance of small differences can be assessed only by statistical treatment of the raw data, which in this case is protein spot intensity and/or area. Therefore, systematic error caused by protein staining should be kept as low as possible. The highly sensitive silver-staining is time-dependent. Therefore, data derived from different gel batches are difficult to compare. Even more difficult is the comparison of data from different laboratories. In contrast, fluorescent staining is an equilibrium reaction, and, being time-independent, more suitable for comparison. For comparison, four 2-D gels (Fig. 7) of urea soluble *E. coli* proteins were stained according to the protocols listed in Tables 1–4.

First, the number of protein spots was compared. 1000 spots were visible by silver-staining, 650 by SYPRO-Ruby staining, 430 by RuBP staining according to Rabilloud *et al.* and 750 spots by the optimized RuBP staining procedure. Second the signal/background ratio was determined. Visual inspection of the four gels shows that the modified RuBP staining affords a picture with high contrast. The intensity of six protein spots (labelled A–F in Fig. 7), selected from the four quadrants of the 2-D gels, was determined as the integral of grey shade *per* scanned line in the profiled area. The background was measured over a protein free area of the same size. Results are shown in Fig. 8A. The signal/background ratios are comparable or better for protein spots stained with the modified RuBP procedure than with any of the other three methods. The next best method was RuBP staining according to Rabilloud *et al.* and SYPRO Ruby staining. Silver-staining was less good.

Thirdly a profile was drawn over a train of three protein spots in the acidic 70 kD region of the gels. The profile boxes are shown in the enlarged region of each gel (Fig. 7). The first protein (Fig. 7A) was identified as chaperone protein Dnak (P04475). The second protein (Fig. 7B) was identified as enzyme I (PtsI, P08839) of the bacterial phosphoenolpyruvate-protein phosphotransferase system. Four Peptides of the third protein matched Dnak.

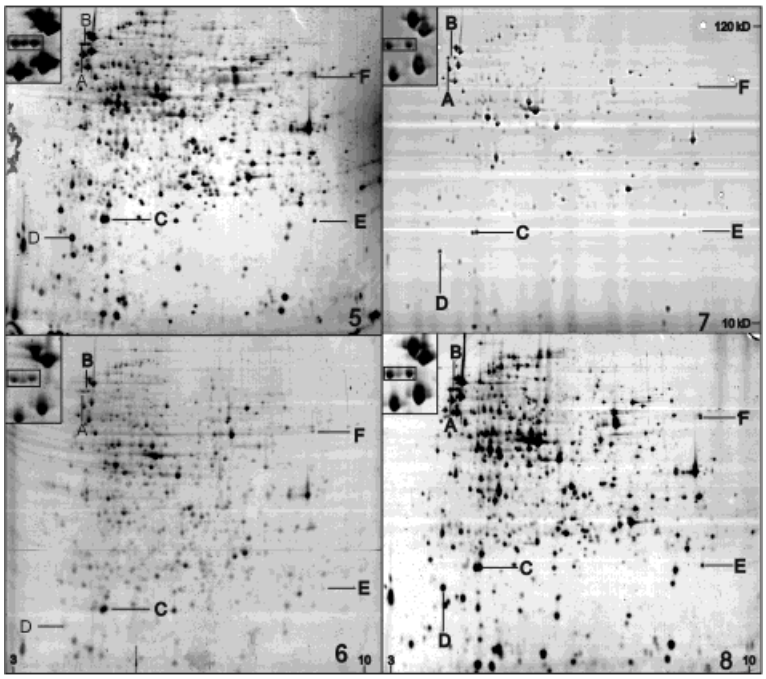

Figure 7. 2-D gels of *E. coli* proteins stained with silver nitrate (5), SYPRO Ruby (6), RuBP according to the procedure of Rabilloud (7), and with RuBP with modifications (8). Eighty μg of protein were loaded *per* gel. For details see Section 3.2. The inserts (top left) show the three spots used for the profile scans shown in Fig. 8A. A–F indicate protein spots used for the calculation of signal/background ratios given in Fig. 4B. A: Dnak, B: PtsI, C: ppa, D: crr, E: Eno, F: Rho.

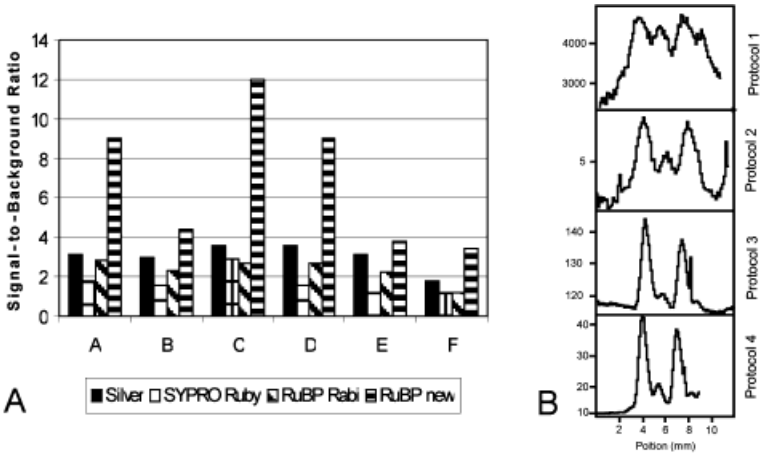

Figure 8. (A) Signal strength-to-background intensity of protein spots A–F from the 2-D gels shown in Fig. 7. Plotted is the ratio of peak intensity/baseline intensity. Intensities in LAU were obtained from scans across entire spots. The proteins were identified by MALDI-TOF. (B) Profile scans over three adjacent protein spots (Dnak, PtsI and unknown), from the 2-D gels shown in Fig. 3., stained with silvernitrate (5), SYPRO Ruby (6), RuBP according to the procedure of Rabilloud *et al.* (7), the modified RuBP procedure (8). Notice differences in peak resolution and signal-to-background intensities.

The identification was not considered safe as it did not reach the value (negative log p = 5) needed to be excluded as a false positive match. The profile scans are shown in Fig. 8B. Silver-staining does not allow for baseline resolution between the three spots. The signal/background ratio for Dnak, the spot on the left, is around 3. Fluorescent staining affords consistently better resolution. SYPRO Ruby staining affords partial, but not baseline resolution and a signal/background ratio of 1.8 for Dnak. RuBP staining according to the procedure of Rabilloud *et al.* and modified as described in this communication gave baseline resolution and signal/background ratios of 2.8 and 9, respectively for Dnak.

Reproducibility was checked by comparison of the spot volumes of six reproduced spots from six independently run and RuBP stained 2-D gels (Table 5). Spot volumes were determined by 2-D densitometry and were then normalized using 2-D Advance Software V6.01 from Phoretix (Nonlinear Dynamics, Durham, NJ, USA). The raw data show SDs of 17 to 31% in spot volume and from 10 to 21% in the normalized data set. The overall SD was 17.4% for the raw data and 15% for the normalized data.

As little as 10 ng of protein *per* spot can be analyzed with the current methods of high-throughput protein identification by MS (Röder, D., F. Hofmann-La Roche, private communication). The sensitivity threshold of RuBP staining is between 6 and 8 ng/spot and is therefore sufficient for the detection of protein spots which can be analyzed by current state-of-the-art technologies.

4 Concluding remarks

The modified RuBP staining is suitable for protein identification by MALDI-TOF. Proteins from spots A (Dnak), C (ppa) and D (IIA^{Gluc}) (Fig. 7) were cut out of 2-D gels giving a total load of 300 μg of *E. coli* protein and stained according to the modified RuBP staining procedure. Proteins were identified *via* peptide mass fingerprinting (data not shown). Detailed studies to compare the sequence coverage with spectra generated by proteins visualized by SYPRO Ruby and the Rabilloud technique are in progress. In conclusion, the modified RuBP staining method has considerable advantages. Staining intensity is linear with protein amount and baseline resolution between

Table 5. 2-D densitometry results

	Gel			Integral/Area-background in (LAU)					AIDA	2-D Advance
Spot	1	2	3	4	5	6	Average	SD	SD in%	SD in%
1	0.35	0.18	0.23	0.2	0.18	0.18	0.22	0.07	30.29	13.94
2	0.31	0.24	0.21	0.24	0.19	0.18	0.23	0.05	20.62	14.48
3	0.18	0.17	0.17	0.23	0.16	0.14	0.18	0.03	17.24	10.35
4	0.31	0.17	0.18	0.23	0.17	0.17	0.21	0.06	27.55	10.10
5	0.27	0.26	0.22	0.21	0.17	0.15	0.21	0.05	22.32	21.87
6	0.3	0.24	0.28	0.24	0.18	0.18	0.24	0.05	20.99	19.10
									Averages	
								0.04	17.37	14.97

closely adjacent protein spots is excellent. The signal-to-background behavior is better than for any of the compared fluorescent staining methods. This fact becomes visible in the excellent contrast of gel images. Good contrast enables protein spots to be detected more easily by image processing software. The method is suitable for staining of analytical gels which can be quantitatively analyzed on a fluorescent scanner or a CCD camera as well as for preparative gels which can be inspected on an UV transiluminator at 254 nm for spot excision. SYPRO Ruby stained gels are known to break (Esquinas, M., Bio-Rad, private communication) easily. This is not the case for RuBP-stained gels. Unlike silver nitrate, which is used at a concentration of 1 g *per* litre, the RuBP concentration needed is only 1 μM. Ruthenium II tris (bathophenantroline disulfonate*)* is not listed as a environmental toxic compound (Bundesamt für Gesundheit Sektion Gewässerschutz, private communication). Last but not least, RuBP is easy to prepare and significantly cheaper to use than other commercially available fluorescent stains.

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