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TECHNICAL NOTE

Stain efficiency and MALDI-TOF MS compatibility of seven visible staining procedures

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Abstract Visible stain is still the most popular protein staining method used in proteomic approaches. However, most published data have been derived from comparisons between visible dyes and fluorescent dyes. In this work, we have focused on seven widely used visible staining procedures—Neuhoff CCB, blue silver, and five silver stains (LKB SN, He SN, Yan SN, Vorum SN, and Blum SN)—and studied their stain efficiencies and MALDI-TOF MS compatibilities on 1-D and 2-D PAGE. It was concluded that blue silver is slightly better in terms of stain efficiency than Neuhoff CCB, but it presented worse MS compatibility. Neuhoff CCB presented better MS compatibility and superior linearity but worse sensitivity than silver stains. Among the five silvering procedures, He SN showed the best MS compatibility and a reasonable staining efficiency; Yan SN lowered the chances of obtaining the protein identity by PMF but gave the best stain efficiency; Vorum SN gave a very clear background and a great contrast, while Blum SN was the worst in this respect. The implications of these results for the selection of a convenient stain are discussed according to specific objectives as well as practical aspects.

Keywords Mass spectrometry · Proteomics · Silver stain · Two-dimensional gel electrophoresis

Abbreviations

CCB	Colloidal Coomassie Brilliant Blue
SN	silver nitrate
TFA	trifluoroacetic acid
HAc	acetic acid

Introduction

Two-dimensional gel electrophoresis (2DE), mass spectrometry (MS) and bioinformatics are the key components of current proteomics technology [1]. Protein visualization on 2DE gels is the key feature of 2DE and MS strongly influences the quality of proteomic analysis. Countless staining procedures have been reported in the literature, and these can be roughly divided into two detection modes: colorimetry and fluorescence [2]. Various fluorescent dyes have recently been developed for protein detection, such as SYPRO Ruby and Deep Purple. These dyes have been shown to combine high sensitivity and compatibility with mass spectrometry [3]. However, they have only found limited use so far, probably due to their high cost and/or technical difficulties.

Coomassie Brilliant Blue (CBB) and silver nitrate (SN) are the most frequently used visible stains. CBB has enjoyed widespread popularity due to its ease of use and compatibility with MS. When formulated as a colloidal sol, Neuhoff's

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colloidal Coomassie Blue G-250 stain (Neuhoff CCB) has a detection limit of about 10 ng of protein per spot [4], and can respond linearly over two orders of magnitude of protein amount (depending on the proteins). Blue silver, a modified Neuhoff CCB, was reported to be more sensitive than Neuhoff CCB, with a detection limit of 1 ng for BSA [5]. Despite the name, it is not as sensitive as silver staining. Silver staining, ever since it was first introduced for protein detection in 1979 [6], has been the most sensitive nonradioactive method for protein visualization, enabling protein spots of just under 0.1 ng to be detected. Though the range of linearity is less than two orders of magnitude, the high sensitivity of silver staining not only lowers the amount of protein needed for proteomic analysis, but it also facilitates the identification of low-abundance proteins. Furthermore, when MS-compatible silvering procedures are used, the protein spots can be visualized without special scanners and subsequently excised from the gel for further MS identification. These advantages have meant that silver staining has remained the most popular detection method for gel-based proteomic analysis.

Today, more than 100 different variants of silver-staining protocols exist for proteins separated in polyacrylamide gels. However, only a few of these have been described as being compatible with protein digestion and MS analysis [7–10]. Selection among these protocols will largely depend on specific needs (efficiency in protein detection, speed, compatibility with subsequent MS analysis). However, most of the information made available to date involves comparing the stain efficiency and/or MS compatibility between visible stains and fluorescent dyes [11–13], with hardly any works examining different visible stains [14]. In this report, using BSA and 293T cell proteins as biological models, we have compared seven different stain procedures—Neuhoff CCB, blue silver, and five popular silver stains (LKB SN [15], He SN [7], Yan SN [8], Vorum SN [9], and Blum SN [10])—with respect to sensitivity, background, dynamic range, and MALDI-TOF MS compatibility.

Experimental

Preparation of protein samples

293T cells were kindly provided by Professor Chen Wang. Cells were harvested, pelleted by centrifugation, and washed twice with ice-cold PBS (pH 7.2). The cell pellets were then solubilized in a lysis buffer containing 7 M urea, 2 M thiourea, 4% CHAPS, 65 mM DTT, and 2% IPGbuffer (pH 3-10NL; Amersham Biosciences, Uppsala, Sweden). After vigorously stirring for one hour, cell debris and insoluble substances were removed by centrifugation at 45 000×*g* for one hour at 10 °C. Protein concentrations were determined using a modified Bradford method [16]. Supernatant was aliquoted at −80 °C until further use.

Gel electrophoresis

Serial dilutions of BSA (Fluka, Buchs, Switzerland) were separated on 12.5% SDS-polyacrylamide minigels of 1 mm thickness using a criterion gel system. For 2-D gels, protein samples of 293T cells were mixed with rehydration solution [8 M urea, 2% CHAPS, 0.4% DTT, and 0.5% IPG buffer (pH 3–10NL)], resulting in a final protein concentration of 130 μg (or 600 μg for preparative gel) in 250 μL. The mixtures were applied onto each 13-cm immobilized pH 3–10 nonlinear gradient dry strip (Amersham Biosciences), and proteins were focused on an IPGphor IEF system (Amersham Biosciences) for a total of 25 kVh (35 kVh for preparative gels). The second-dimension separation was performed on 12.5% polyacrylamide gels (260 mm× 200 mm×1 mm) with an Ettan DALT Twelve apparatus (Amersham Biosciences). A mixture of recombinant proteins was used as molecular weight standards.

Staining and image acquisition

For CCB, gels were stained following the classical method of Neuhoff et al [4], or stained with blue silver, which has a 20% increase in dye and a five-fold increased concentration of phosphoric acid compared to the original Neuhoff CCB [5]. For SN, gels were stained according to five different procedures, as shown in Table 1 [7–10, 15]. Stained gels were digitized using a D2000 Uniscan scanner (Tsinghua Uniscan, Beijing, China).

Tryptic in-gel digestion

Spots were excised from the 1DE or 2DE gels. The in-gel digestion method reported by Shevchenko was performed as described in [17] with some modifications. Briefly, gel spots were destained with 50% (v/v) ACN and 50 mM NH_4HCO_3 for CCB stained spots or with a 1:1 solution of 30 mM $K_3Fe(CN)_6$ and 100 mM $Na_2S_2O_3.5H_2O$ for silver-stained spots. After reducing in 10 mM DTT in 50 mM NH_4HCO_3 for 1 h at 56 °C and alkylating in the same volume of 55 mM iodoacetamide in 50 mM NH_4HCO_3 for 45 min at ambient temperature in the dark, the gel pieces were rehydrated on ice for 45 min in 2.5 μL of 25 mM NH_4HCO_3 containing 10 ng/μL sequencing-grade trypsin (Promega, Madison, WI, USA). Proteins were digested overnight at 37 °C. Peptides were sequentially extracted with 5% (v/v) trifluoroacetic acid (TFA) and 2.5% (v/v) TFA/ 50% (v/v) ACN. Supernatants were pooled and dried in a speed-vac. Peptides were then resuspended in 1.5 μL of 0.5% (v/v) TFA.

Table 1 The different silver staining procedures assessed

Step	LKB SN	He SN	Yan SN	Vorum SN	Blum SN
1. Fix	50% EtOH, 10% HAc; overnight	40% EtOH, 10% HAc; overnight	40% EtOH, 10% HAc; overnight	50% MeOH, 12% HAc, 0.05% formalin; overnight	40% MeOH, 10% HAc; overnight
2. Wash	–	–	–	35% EtOH; 20 min×3	30% EtOH; 20 min×2. H_2O; 20 min
3. Sensitize	30% EtOH, 0.25% glutaraldehyde, 6.8% NaAc, 0.2% $Na_2S_2O_3$; 30 min	30% EtOH, 4.1% NaAc, 0.2% $Na_2S_2O_3$; 30 min	30% EtOH, 6.8% NaAc, 0.2% $Na_2S_2O_3$; 30 min	0.02% $Na_2S_2O_3$; 1 min	0.02% $Na_2S_2O_3$; 1 min
4. Wash	H_2O; 5 min×3	H_2O; 5 min×3	H_2O; 5 min×3	H_2O; 3 min×2	H_2O; 20 s×3
5. Silvering	0.1% $AgNO_3$, 0.02% formalin; 20 min	0.1% $AgNO_3$, 0.02% formalin; 40 min	0.25% $AgNO_3$; 20 min	0.2% $AgNO_3$, 0.076% formalin; 20 min	0.1% $AgNO_3$; 20 min; 4 °C
6.Wash	H_2O; 1 min×2	H_2O; 1 min×2	H_2O; 1 min×2	H_2O; 20 s×2	H_2O; 20 s×3
7. Develop	2.5% Na_2CO_3, 0.01% formalin	2.5% Na_2CO_3, 0.01% formalin	2.5% Na_2CO_3, 0.04% formalin	6% Na_2CO_3, 0.05% formalin, 0.0004% $Na_2S_2O_3$	3% Na_2CO_3, 0.05% formalin
8.Wash	–	–	–	–	H_2O; 20 s
9. Stop	1.46% EDTA; 10 min	1.46% EDTA; 10 min	1.46% EDTA; 10 min	50% MeOH, 12% HAc; 5 min	5% HAc; 10 min
10. Wash	H_2O; 5 min×3	H_2O; 5 min×3	H_2O; 5 min×3	H_2O; 5 min×3	H_2O; 5 min×3

MALDI-TOF MS and protein identification

Digested peptide samples were co-crystallized with an equal volume of saturated matrix solution, α-cyano-4-hydroxy cinnamic acid in 0.1% TFA in H_2O/ACN (2:1), on the MALDI target. MS spectra were obtained using an ABI 4700 proteomics analyzer mass spectrometer (Applied Biosystems, Framingham, MA, USA). Six external standards (Applied Biosystems) were used to calibrate each spectrum, and the max. outlier error was set at 5 ppm in MS reflector positive operating Mode. An MS database search was performed using GPS Explorer™ software (version 3.5; Applied Biosystems) and MASCOT software (version 2.0; Matrix Science, London, UK). Searches were performed with carbamidomethylation of cysteine and oxidation of methionine residues as variable modifications. Peptide mass tolerance and fragment mass tolerance were set to 50 ppm and ± 0.1 Da, respectively. Peptide mixtures that yielded statistically significant search scores (> 95% C. I., equivalent to a MASCOT expect value of < 0.05) and accounted for the majority of the ions present in the mass spectra were defined as positive identifications.

Data analysis

Gel images were analyzed using ImageMaster™ 2D Platinum software (version 5.0; Amersham Bioscience). Protein bands or spots were detected automatically. Manual spot editing or deleting (of artifacts) was performed when necessary. The amount of each protein band or spot was expressed as its volume, which was calculated as the volume above the spot border and situated at 75% of the spot height (measured from the peak of the spot). The MS spectra obtained were analyzed using Data Explorer (version 4.3; Applied Biosystems). S/N values for five BSA-specific peaks of varying intensity were calculated using the in-built S/N calculator function. A Wilcoxon paired-sample test was performed on the PMF scores using the statistiXL software (version 1.6).

Results

Comparative background and sensitivity

Well-resolved patterns were observed for the seven staining procedures on the 1DE and 2DE gels (see Figs. 1 and 2). Direct inspection of the gel images showed substantial differences concerning the background. Vorum SN gave a very clean background and a very clear contrast, while Blum SN gave the worst background and contrast. The other three SN gave similar backgrounds and contrasts. In the case of CCB, a rough contrast and a clear background was obtained at the expense of sensitivity.

The sensitivities of various visible stains were assessed by searching for the minimal amount of BSA that was detectable in SDS-PAGE (Fig. 1) and by comparing the number of spots detected in 2DE gels. In SDS-PAGE gels, LKB SN, He SN and Yan SN appeared to be able to detect BSA down to 0.5 ng, whereas the two other silver stains required about 1 ng. At the same time, this pattern was detected for about 8 ng of protein with Neuhoff CCB or blue silver. The 2DE gels were loaded with identical quantities of protein (approximately 130 μg). Among the stains tested, silver stains detected approximately five times the number of protein spots that CCB did. LKB SN and Yan SN were the most sensitive, with average detections of 1318 and 1313 spots per gel, respectively. Vorum SN only detected 1150 spots. Neuhoff CCB detected 232 spots, and a large number of low-abundance proteins were only visualized when the loading amount was increased to 600 μg (1120 spots were detected; also see Fig. 2).

Linearity analysis

In order to get information on the linearity of response, the volume of BSA from Fig. 1 was measured according to the total amount of proteins loaded per lane. It is clearly apparent from Fig. 3 that different SN procedures differed greatly in linearity, with regression coefficients ranging between 0.844 and 0.965, while Neuhoff CCB and Blue silver provided better linearity, with regression coefficients of 0.988 and 0.991 respectively. A propensity to saturate was observed for Vorum SN.

Differential staining of proteins

In this study, the differential staining of a significant number of proteins was observed, as exemplified in Figs. 1 and 2. This phenomenon was also observed in the uneven staining of the proteins from different molecular weight ranges. As showed in Fig. 1, besides the band of BSA, several other protein bands were also visualized, but with different intensities. In the 2DE gels (Fig. 2b), while the seven procedures stained proteins of high and middle molecular weight ranges with similar efficiencies, SN stained proteins of low molecular weight much more efficiently than the CCB stains, especially when the LKB SN and He SN procedures were used.

MALDI-TOF MS compatibility

To obtain a clear picture of the influence of the seven visible stains on the quality of the MALDI MS analysis,

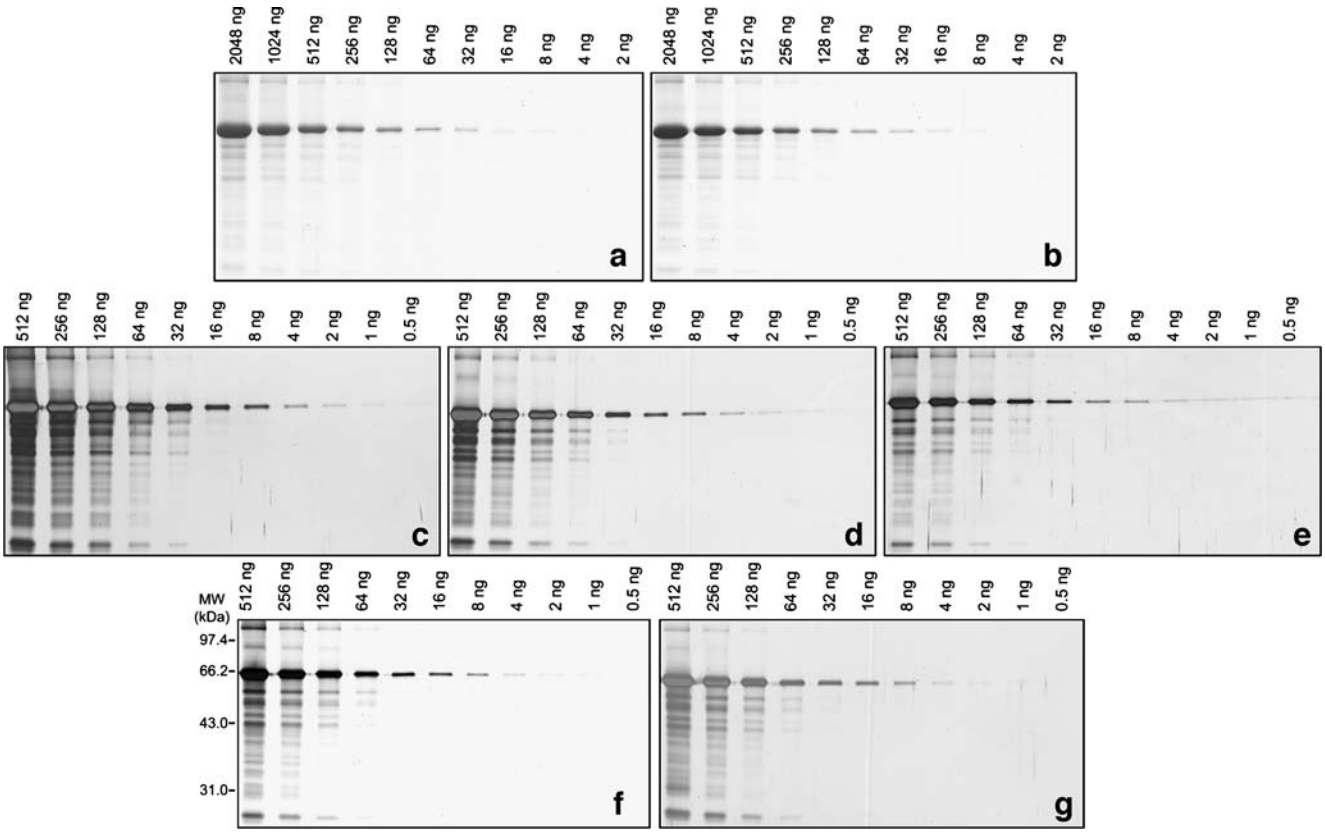

Fig. 1 Serial dilutions of BSA (66 kDa) run on SDS-PAGE minigels. Gels were stained with (**a**) Neuhoff CCB, (**b**) blue silver, (**c**) LKB SN, (**d**) He SN, (**e**) Yan SN, (**f**) Vorum SN, or (**g**) Blum SN. The protein amounts indicated apply to BSA

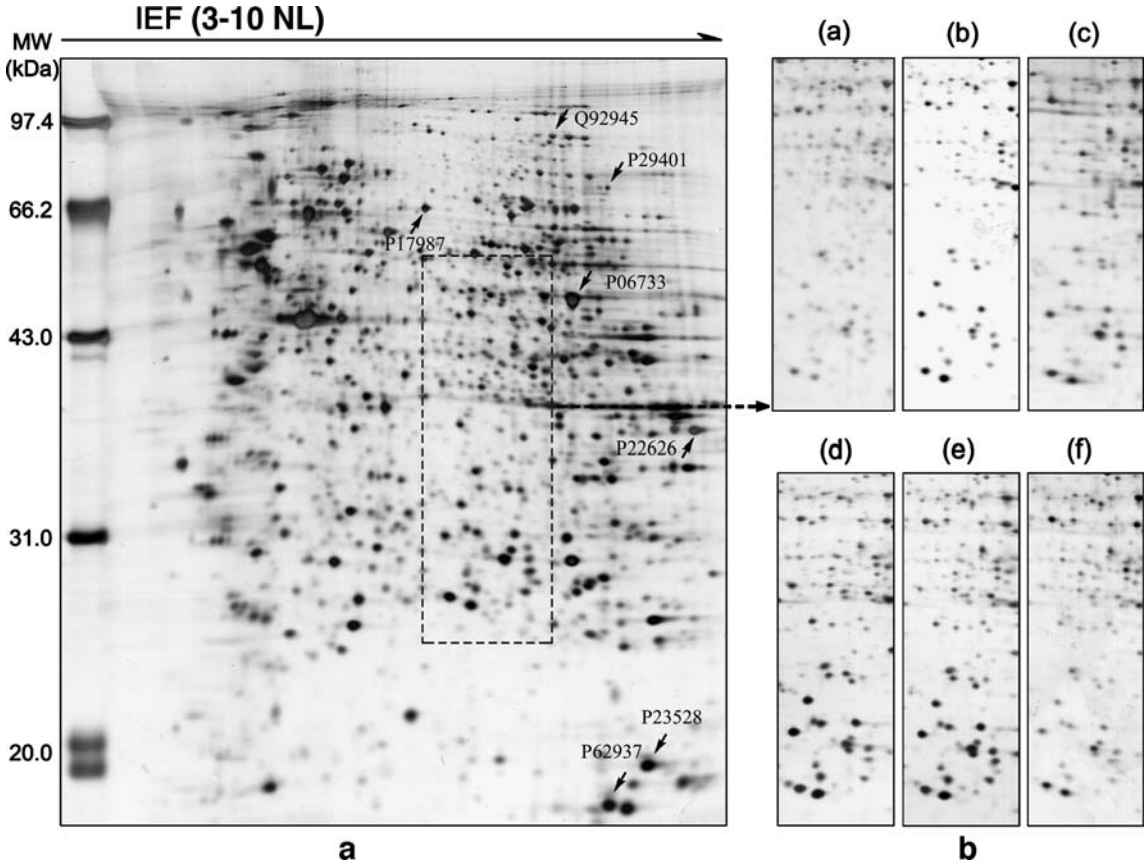

Fig. 2 Comparative staining of 293T cell proteins. **a** 2-D PAGE gel of 293T cell proteins with *arrows* indicating the locations of proteins identified by PMF. The gel shown was loaded with 200 μg of total protein and stained using Yan SN. **b** Differential staining of proteins with (*a*) Neuhoff CCB, (*b*) Vorum SN, (*c*) Blum SN, (*d*) LKB SN, (*e*) He SN, and (*f*) Yan SN. Gels were loaded with 130 μg of total protein, except for the Neuhoff CCB-stained gel, which was loaded with 600 μg of protein

spots (1 mm in diameter) excised from two lanes (loaded with 64 ng and 16 ng of BSA respectively) of the 1-D gels were submitted to MALDI-TOF MS analysis. In addition, a set of 2DE separations of a protein mixture from 293T cells was included. 151 gel pots, corresponding to seven proteins which were selected stochastically (indicated in Fig. 2a), were excised from the reduplicate gels and analyzed by MALDI-TOF MS. Acquired MS of the tryptic peptides were analyzed in terms of S/N for protein-specific peaks of various masses and intensities, sequence coverages, and Mascot scores. Identified 293T cell proteins are summarized in Table 2.

Upon reviewing the acquired mass spectra of BSA (Fig. 4), it was found that LKB SN, Yan SN, and Blum SN exhibited some similarity in terms of their impacts on MALDI-TOF MS (Fig. 5). This similarity can also be observed in the comparison between the scores for BSA and those for five other 293T cell proteins (P17987, P23528, Q92945, P29401, and P62937), which gave lower values than obtained for other silver stains (Fig. 6c,d). As is evident from Fig. 6a, blue silver presented worse MS compatibility than the traditional Neuhoff CCB. At the same time, the LKB SN-stained samples gave the roughest results. The data also showed that Neuhoff CCB- and He SN-stained samples gave superior S/N-values compared to the other stains. This superiority was further confirmed by the Mascot scores of PMF (Fig. 6c,d). However, in terms of sequence coverage, the superiority disappeared when the protein amount was greatly reduced (Fig. 6b). A Wilcoxon paired-sample test was performed on the Mascot scores for the identified proteins. The resulting *p*-values presented in Table 3 indicate that Neuhoff CCB was superior to blue silver in terms of MALDI-TOF MS identification (p=0.004). He SN exhibited

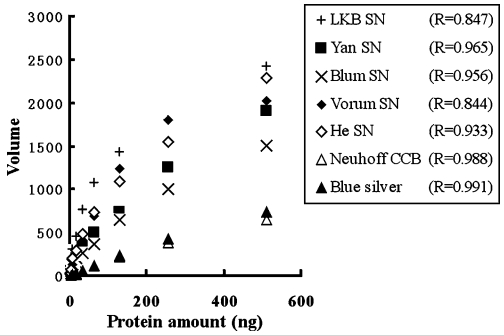

Fig. 3 Relationship between band volume and protein amount. *Insets*: correlation coefficients between band volume and protein amount

Table 2 293T cell proteins identified by MALDI-TOF MS

Protein name	Acc. no.[a]	Theor. M_r (Da)/pI[b]	Average sequence coverage (%)						
			LKB SN	Yan SN	Vorum SN	Blum SN	He SN	Neuhoff CCB	Blue silver
Far upstream element-binding protein 2	Q92945	72664/8.02	18	23	33	29	29	29	26
Transketolase	P29401	67835/7.58	20	21	22	21	30	34	23
T-complex protein 1 subunit alpha	P17987	60306/5.8	26	21	37	36	38	47	37
Alpha-enolase	P06733	47139/7.01	69	67	62	74	62	68	68
Heterogeneous nuclear ribonucleoproteins A2/B1	P22626	37407/8.97	61	65	58	69	75	71	71
Cofilin-1	P23528	18491/8.22	33	39	42	38	75	65	54
Peptidyl-prolyl *cis-trans* isomerase A	P62937	17870/7.82	29	40	35	35	35	50	47

[a] *Acc. no*, Swiss-Prot database accession number
[b] *Theor. Mr(Da)/pI*, theoretical molecular weight of the matched protein in Da /theoretical isoelectric point of the matched protein

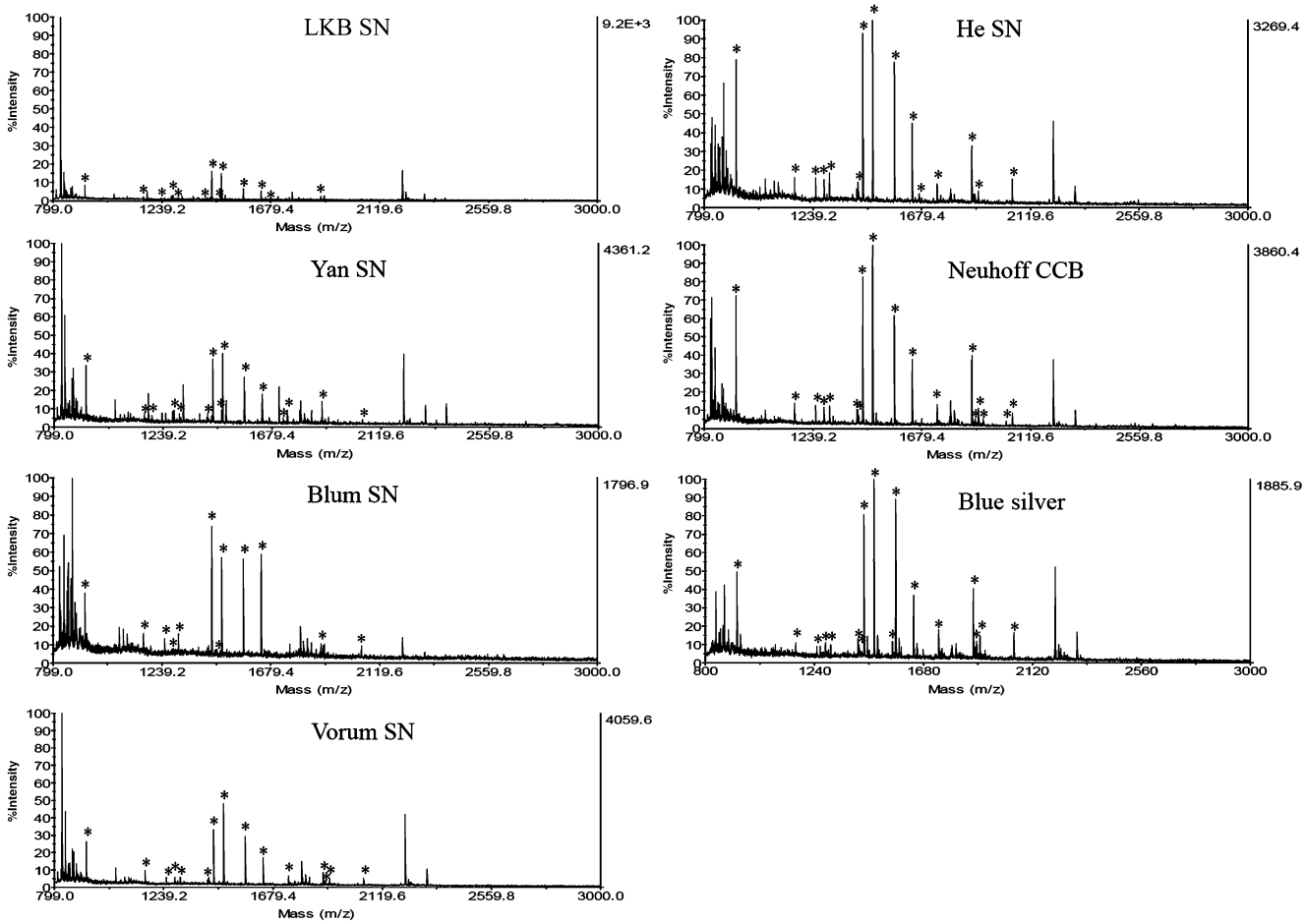

Fig. 4 MALDI-TOF mass spectra for replicate spots in SDS-PAGE gels loaded with 16 ng of BSA and stained with LKB SN, Yan SN, Blum SN, Vorum SN, He SN, Neuhoff CCB, or blue silver. Peaks that matched the database entry for BSA are marked with asterisks

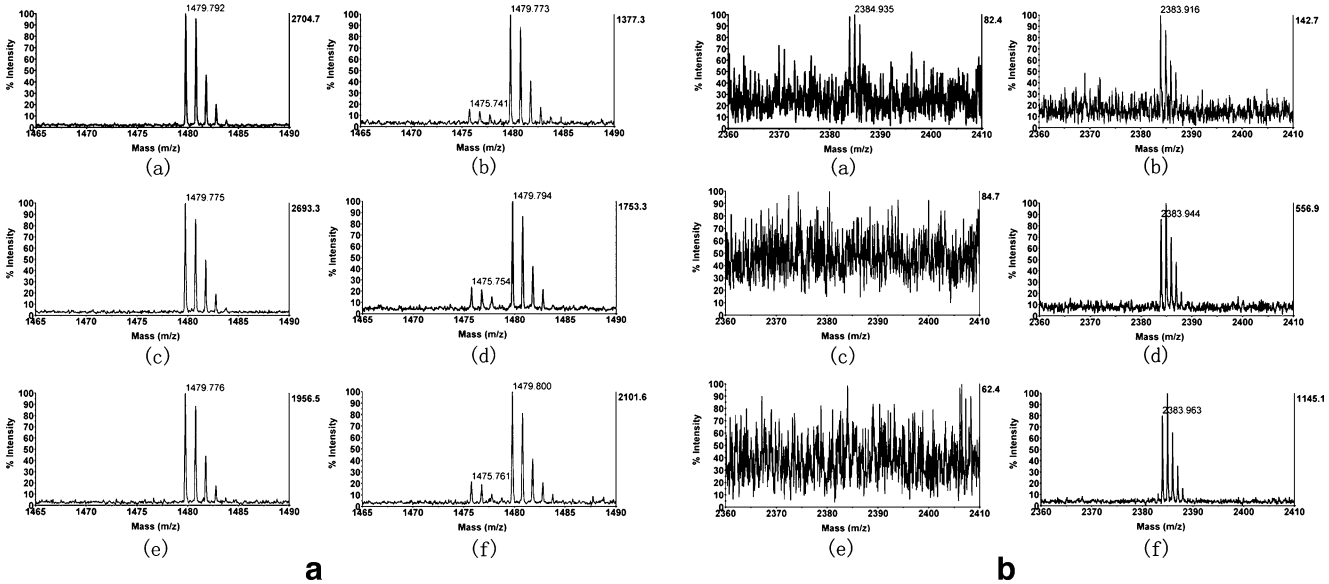

Fig. 5 Comparison of MALDI-TOF MS of two peptides, *m/z* 1475.7 (**a**) and *m/z* 2383.9 (**b**) obtained by the tryptic in-gel digestion of BSA, which were separated by SDS-PAGE and then stained with (*a*) Neuhoff CCB, (*b*) LKB SN, (*c*) He SN, (*d*) Yan SN, (*e*) Vorum SN, or (*f*) Blum SN. The subsequent PMF showed that the peptide at *m/z* 1475.7 matched with BSA while the peptide at *m/z* 2383.9 did not

superiority over other selected silvering procedures ($p<0.05$ except Yan SN, whose $p=0.055$). It was difficult to figure out the superiority between Yan SN and Blum SN ($p=1$). Also, it was difficult to define the superiority between Vorum SN and blue silver ($p=0.938$).

Discussion

In this study, to assess the stain quality and compatibility of visible stains with MALDI-TOF MS, we selected seven procedures that have been widely used. Due to its high sensitivity compared to CBB R250 and its excellent MS compatibility, Colloidal Coomassie Blue G-250 stain is the most popular CBB stain used in proteomic approaches. Neuhoff CCB has been cited more than one thousand times since its publication in 1988 [4]. Blue silver is the modified Neuhoff CCB stain by Candiano and his colleagues. It was reported to be more sensitive than Neuhoff CCB, and has been cited 90 times since 2004 [5]. Among the selected silvering procedures, LKB SN, He SN and Yan SN are modified methods based on the procedure of Heukeshoven et al. LKB SN was the method optimized by Pharmacia LKB [15, 18]. It includes glutaraldehyde in the sensitization solution and is noted as being a MS incompatible method. However, due to its high sensitivity, LKB SN is still a popular silvering method in analytical gels. Yan SN and He SN omit the glutaraldehyde in the sensitization solution, and are described as MS-compatible methods. Yan SN has been cited 178 times since 2000, and is available as a commercial kit (Silver Stain PlusOne; Amersham Pharmacia Biotech, Amersham, UK) [8]. He SN is a method that was optimized by He et al., and has been adopted in several publications [19–21]. Though it is not as popular as Yan SN, it shows many similarities with LKB SN and Yan SN (see Table 1). A comparison of these procedures may not only be helpful to guide the selection of staining procedures in proteomic approaches, but it can also lead to insights into the mechanism of the protein staining. Blum SN is a well-established silver staining procedure. It has been cited thousands of times since its publication in 1987 [10]. Vorum SN shows some similarities with Blum SN. It was presented at the 48th American Society for Mass Spectrometry Conference on Mass Spectrometry and Allied Topics, 11–15 June 2000, Long Beach, CA.

Uneven staining intensity is commonly observed for the same amount of protein, and this can be misleading for biological samples that contain complex mixtures of very different amounts of proteins and for which detection by pattern is of interest, as the results for the BSA and 293T cell proteins showed.

A recent paper has studied the compatibility with ESI MS of several visible stains, including Yan SN, Blum SN and Neuhoff CCB, and different results were obtained [14]. This may result from the different impacts of a certain staining procedure on different ionization modes. The data presented in this study suggest that staining gels with Neuhoff CCB offers several advantages over silver staining for proteomic projects. Since the linear dynamic range of Neuhoff CCB is better than that of silver staining, quantitative differences in protein expression are easier to detect in 2DE gel images. Moreover, Neuhoff CCB presents superior compatibility with MALDI-TOF MS. However, the sensitivity of Neuhoff CCB is much lower

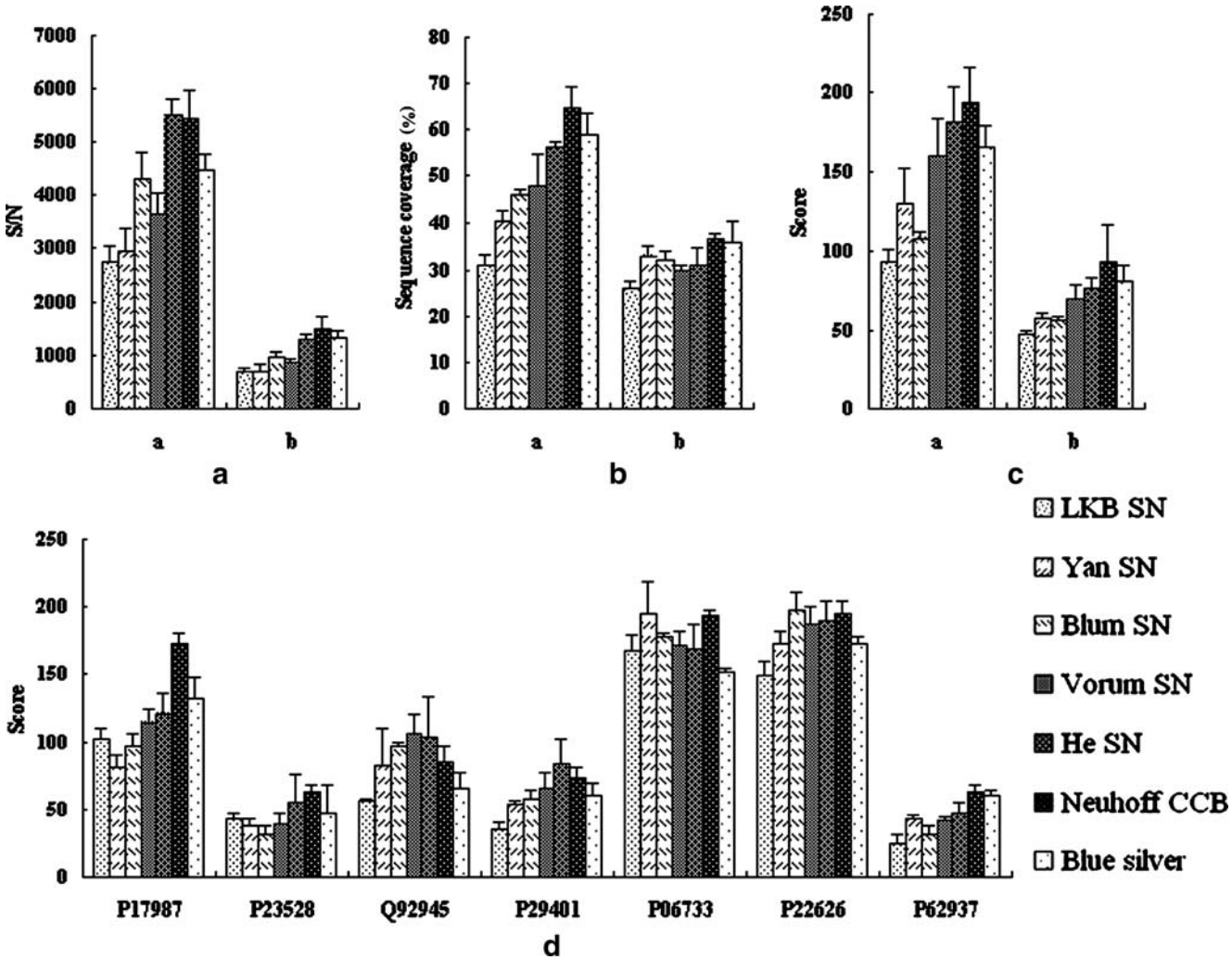

Fig. 6 Effect of visible stains on MALDI-TOF MS of BSA and 293T cell proteins. **a** Signal-to-noise values (S/N) for five BSA-specific peaks (927.49, 1439.81, 1479.80, 1567.74, and 1880.92) were calculated. Spots from two BSA bands (*a*: loaded with 64 ng of BSA; *b*: loaded with 16 ng of BSA) in SDS-PAGE gels stained with seven different visible stains were tryptic digested and analyzed by MALDI-TOF MS. **b** The values for sequence coverage of BSA. **c** The values for the Mascot score of BSA. **d** The values for the Mascot score sof 293T cell proteins separated by 2DE. Mascot scores were taken from the search results using GPS Explorer™ software. In this program, a Mascot score of >53 was considered significant ($p<0.05$). Each experiment was performed at least three times. Histograms represent the average value ± S.D.

than silver staining. Though high protein loading may result in the visualization of low-abundance proteins, it can also bring about many problems, such as difficult protein separation in 2DE gels. Thus, Neuhoff CCB is a good choice of preparative gel. Blue silver stain gels much more efficiently than Neuhoff CCB, but it gives rougher results in MALDI-TOF MS analysis, and doesn't address the problem with sensitivity.

The difference in stain efficiency and MS compatibility between SN and CCB mainly comes from the interaction mechanisms. For instance, the binding of CBB G-250 to proteins is attributed to van der Waals forces and hydrophobic interactions involving argininyl residues, rather than other basic residues or aromatic residues [22–24]. For silver nitrate, the staining relies on salt or complex formation

Table 3 Wilcoxon paired-sample test of Mascot scores of identified proteins

	LKB SN	Yan SN	He SN	Vorum SN	Blum SN	Neuhoff CCB
Yan SN	0.055					
He SN	0.004	0.055				
Vorum SN	0.012	0.129	0.020			
Blum SN	0.055	1.000	0.031	0.055		
Neuhoff CCB	0.004	0.008	0.164	0.023	0.020	
Blue silver	0.020	0.220	0.098	0.938	0.426	0.004

Values of p <0.05 were considered statistically significant

involving sulfhydryl and carboxyl groups of amino acid side chains [25]. Since the five SN stains share very similar interaction mechanisms, the differences between them are apparently related to the recipe in each step or the details of each procedure.

Glutaraldehyde, which is known to attach covalently to the protein through Schiff base formation with ε- and α-amino groups [25], was also used as sensitizer to enhance the contrast between the stained protein bands and the background, thus improving the sensitivity. However, it also results in the covalent modification of proteins, and interferes with peptide identification in MS. This adverse impact on MS was observed in our data (Fig. 6). However, according to our results, the glutaraldehyde sensitization step is not crucial for obtaining detection limits in the low nanogram range (Fig. 1), which is also in agreement with previous observations [26]. Formaldehyde serves as the reductant to convert silver ion to metallic silver at high pH. The interference between silver staining and MS has been investigated in detail [27], and an interference mechanism in which protein crosslinks with formaldehyde in alkaline media has been proposed to be the major phenomenon that takes place. Previous studies suggested omitting formaldehyde in the silver stain solution in order to improve MS identification. However, in view of the results for He SN, Yan SN, and Blum SN (see Fig. 6 and Table 3), this seems to have no effect, which is probably due to the rise in the concentration of formaldehyde in developing solutions. Since silver staining stains proteins in the low molecular weight range much more efficiently, protein crosslinking seems to be beneficial for silvering proteins with low molecular weights. Including formaldehyde in the silver stain solution seems to promote this effect, because this phenomenon was much more evident when LKB SN and He SN were used.

In conclusion, the present work aimed at guiding the selection of staining procedures in proteomic approaches. Neuhoff CCB shows better compatibility with MALDI-TOF MS and superior linearity, but inferior sensitivity as compared to silvering. It may be a good choice in preparative gels. Among the five silvering procedures, He SN shows the best compatibility with MS and a reasonable staining efficiency. It is a good choice for proteomic analysis. Yan SN lowers the chances of obtaining the protein identity by PMF. However, due to the superior linear response curve of this stain, its sensitivity as well as its ease of handling, Yan SN still is a good choice for 2DE gel staining aimed at protein-expression profiling. However, one must note that it may be necessary to stain gels with Neuhoff CCB in parallel in order to improve the chances of obtaining protein identification. When the quality of the protein patterns is important, Vorum SN may be a good choice due to its clear background and great contrast.

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