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Chromium (III) enhanced diamine silver staining of proteins and DNA in gels

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Abstract

Chromium (III) enhanced the sensitivities of diamine silver staining of four proteins between 6- and 50-fold over that of the Coomassie Brilliant Blue (CBB)-chromium modified thiosulfate-silver staining method (Zhou *et al*. *Biotechnology Letters*, 2002, **24**: 1561–1567). Using six dsDNA fragments, the detection limits of this new method was 10 to 30 pg per band, being 10- to 25-fold more sensitive than previous methods.

Introduction

Silver staining can detect proteins, nucleic acids, sialoglycoproteins and lipids in polyacrylamide gels (Dzandu *et al*. 1984, Ansorge 1985, Merril 1990, Bassam *et al*. 1991). Recently we reported the use of chromium (III) as an enhancer for silver staining of proteins in gels (Zhou *et al*. 2002). In this paper, we show that chromium (III) can further improve the sensitivities of diamine silver staining of proteins and DNA in gels. Of the four proteins studied, we found the detection limits of the Coomassie Brilliant Blue (CBB)-chromium modified diamine silver staining of proteins to be about 3 to 83 pg per band. Its maximal sensitivity was about 6- to 50-fold greater than that of the published sensitive CBB-chromium (III) modified thiosulfate-silver staining method (Zhou *et al*. 2002). Of the six dsDNA fragments studied, the detection limits of the chromium (III) modified diamine silver staining of DNA were about 10 to 30 pg per band. Its maximal sensitivity was about 10- to 25-folds over that of the published diamine silver staining method (Vari & Bell 1996).

Materials and methods

Materials

The six proteins used in this study were products of Shanghai Li-Zhu-Dong-Fong Biotech. They were rabbit phosphorylase b (97 000) bovine serum albumin (66 200), rabbit actin (43 000), bovine carbonic anhydrase (31 000), trypsin inhibitor (20 000) and hen egg white lysozyme (14 400). They were dissolved in loading buffer, heated at 95 °C for 3 min and subjected to electrophoresis. The six dsDNA fragments used in the study were fragments of DNA ladder from TaKaRa (product name: DNA marker DL 2000). It contained dsDNA fragments of 2000 bp, 1000 bp, 750 bp, 500 bp, 250 bp and 100 bp. The concentration of each dsDNA fragment was about 10 ng μl^{-1}. All chemicals used were of analytical grade and double distilled water was used for preparation of buffers and gels.

Gel electrophoresis

SDS-PAGE of the six proteins was carried out in vertical polyacrylamide gels (10 × 8 × 0.12 cm) with

1.5 cm 4% (w/v) stacking gel on top of 6.5 cm 12.5% (w/v) separation gel. Electrophoresis was carried out toward anode at 60 V in 4% (w/v) stacking gel and 120 V in 12.5% (w/v) separation gel until the tracking dye touched the bottom of the glass plate. Electrophoresis of the six dsDNA fragments was carried out vertically at 80–100 V in 5% polyacrylamide gel.

Silver staining of dsDNA fragments and proteins in gels with previous published and modified diamine silver staining methods

The detailed procedures of published and modified diamine silver staining of proteins and dsDNA in gels are illustrated in Table 1.

Analysis of sensitivity enhancements of respective modified diamine silver staining methods

Among the six proteins used, detection limits of rabbit actin (43 000), bovine carbonic anhydrase (31 000), trypsin inhibitor (20 000) and hen egg white lysozyme (14 400) were carefully studied to show exact sensitivity enhancement between the new methods and previously published method. Results from the four proteins studied using the Coomassie Brilliant Blue (CBB)-chromium (III) modified diamine silver staining of proteins was compared with that of the published sensitive CBB-chromium (III) modified thiosulfate-silver staining (Zhou *et al*. 2002) to show the further enhancement of sensitivity. Of the six dsDNA fragments studied, the maximal sensitivity of the chromium (III) modified diamine silver staining was compared with that of the published diamine silver staining (Vari & Bell 1996). For both sets of comparisons, the extents of sensitivity enhancement were calculated according to their respective detection limits. Experimental results were obtained from at least five replicates. Significance of difference was statistically analyzed with *t*-test. Difference would be considered to be significant, if $p < 0.05$.

Results and discussion

Silver staining procedures were characterized into two groups: acidic silver staining procedures and basic silver staining procedures (diamine silver stainings) (Rabilloud 1990). We have recently shown that chromium (III) is a new sensitivity enhancer for silver staining of proteins in gels (Zhou *et al*. 2002). The sensitivity of the thiosulfate-silver staining of proteins

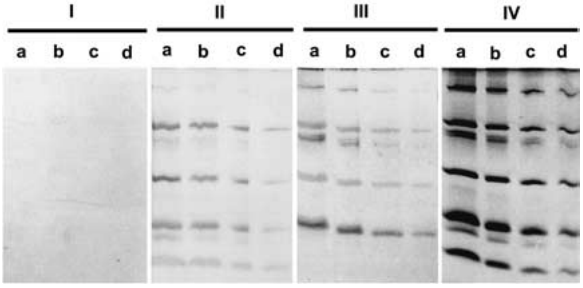

Fig. 1. The sensitivity of diamine silver staining of proteins was enhanced with CBB-R 250 and/or Cr (III). The six proteins (from top to bottom) were rabbit phosphorylase b (97 000) bovine serum albumin (66 200), rabbit actin (43 000), bovine carbonic anhydrase (31 000), trypsin inhibitor (20 000) and hen egg white lysozyme (14 400). The loading amount of each protein per band was as follows: lane a, 100 ng; lane b, 50 ng; lane c, 25 ng; lane d, 12.5 ng, respectively. I, the published diamine silver staining of proteins (Merril *et al*. 1998), II, the CBB modified diamine silver staining of proteins, III, the Cr (III) modified diamine silver staining of proteins, IV, the CBB-Cr (III) modified diamine silver staining of proteins. The developing time was 3 min.

in gels, an acidic silver staining procedure, could be enhanced by the chromium (III) and CBB (Zhou *et al*. 2002). Here we extended our study and found that the sensitivity of diamine silver staining of proteins in gels could also be enhanced by the chromium (III) and CBB (Figure 1 and Table 2). Chromium (III) and CBB could facilitate the developing of gels, improve the contrast of stain and enhance maximal sensitivity (Figure 1 and Table 2). Among the four published and modified diamine silver staining procedures for staining proteins in gels, our CBB-chromium modified diamine silver staining of proteins has the highest sensitivity (Figure 1 and Table 2), and was also higher than that of the published sensitive CBB-chromium modified thiosulfate-silver staining method (Table 2). Of the four proteins studied, the detection limits of the new method reached 3 to 83 pg per band. Its maximal sensitivity was about 6- to 50-fold over that of our previous published sensitive CBB-chromium modified thiosulfate-silver staining (Table 2). For example, the detection limit of rabbit actin was about 50 pg per band using the previous method (Zhou *et al*. 2002). However, the detection limit of the same protein using the new method was about 3 pg per band (Table 2), which was about 17 more folds over that of previous method.

We also found that the chromium (III) could enhance the sensitivity of various acidic and basic silver staining procedures for silver staining of dsDNA fragments in gels (data not shown). Vari & Bell (1996)

Table 1. Procedures of the published and modified diamine silver staining methods.

	Silver staining procedures	I	II	III	IV	V	VI
Steps	Reagents	Time (min)					
Fix	25% (w/v) trichloroacetic acid	30	30	30	30		
Rinse	25% (v/v) 2-propanol, 5% (v/v) acetic acid			15 × 2			
Staining	*0.25% (w/v) CBB R-250*[a], 45% (v/v) methanol, 10% (v/v) acetic acid		240		240		
Destaining	45% (v/v) methanol, 10% (v/v) acetic acid		overnight		overnight		
Rinse	10% (v/v) ethanol, 5% (v/v) acetic acid	180	10	10	10		
Rinse	Double distilled water	10	10	10	10	20 × 3	20 × 3
Sensitize	*12% or 2% (w/v) glutaraldehyde*	30 (12%)	30 (12%)	30 (12%)	30 (12%)	30 (2%)	30 (2%)
Rinse	Double distilled water	10 × 3	10 × 3	10 × 3	10 × 3	15 × 2	15 × 2
Rinse	Double distilled water	30 × 4	30 × 4	30 × 2	30 × 2		
Sensitize	*0.2% (w/v) potassium chromium sulfate*			10	10		10
Rinse	Double distilled water			10 × 2	10 × 2		10 × 2
Rinse	Double distilled water			30 × 1	30 × 1	1080	1080
Stain	*Silver diamine solution a or b*[b]	20 (a)	20 (a)	20 (a)	20 (a)	20 (b)	20 (b)
Rinse	Double distilled water	1/4 × 2	1/4 × 2	1/4 × 2	1/4 × 2	10 × 3	10 × 3
Rinse	Double distilled water	5 × 2	5 × 2	5 × 2	5 × 2		
Rinse	Double distilled water	10 × 1	10 × 1	10 × 1	10 × 1		
Develop	Developing solution a or b[c]	5–30 (a)	5–30 (a)	5–30 (a)	5–30 (a)	5–30 (b)	5–30 (b)
Stop	5% (v/v) acetic acid	5	5	5	5	5	5

[a]CBB R-250; Coomassie Brilliant Blue R-250.
[b]Silver diamine solution a: 0.8% (w/v) silver nitrate, 2.8% (v/v) ammonia, 50 mM sodium hydroxide; silver diamine solution b: 0.4% (w/v) silver nitrate, 1% (v/v) ammonia, 50 mM sodium hydroxide. They were made by combining a silver nitrate solution with an ammonium/sodium hydroxide solution. Prepared just prior to use.
[c]Developing solution a: 0.01% (w/v) citric acid, 0.006% (v/v) formaldehyde; developing solution b: 0.005% (w/v) citric acid, 0.05% (v/v) formaldehyde; I, the published diamine silver staining of proteins (Merril *et al.* 1998); II, the CBB modified diamine silver staining of proteins; III, the Cr (III) modified diamine silver staining of proteins; IV, the CBB-Cr (III) modified diamine silver staining of proteins; V, the published diamine silver staining of DNA (Vari & Bell 1996); VI, the Cr (III) modified diamine silver staining of DNA. These procedures were suitable for gels with 1 mm thick.

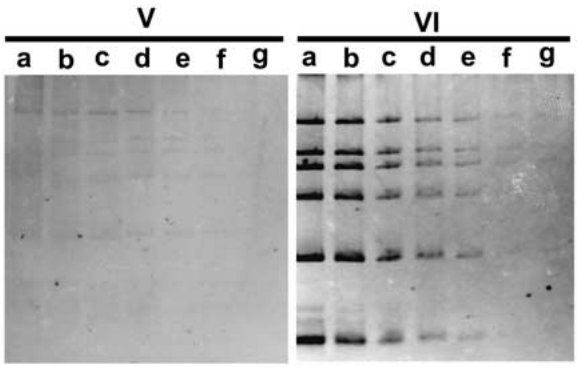

Fig. 2. The sensitivity of diamine silver staining of dsDNA fragments was enhanced with the Cr (III). The dsDNA fragments (from top to bottom) were 2000 bp, 1000 bp, 750 bp, 500 bp, 250 bp and 100 bp respectively. The loading amount of each dsDNA fragment per band was as follows: lane a, 50 ng; lane b, 25 ng; lane c, 10 ng; lane d, 5 ng; lane e, 2.5 ng; lane f, 1 ng, lane g, 0.5 ng respectively. V, the published diamine silver staining of DNA (Vari & Bell 1996). VI, the Cr (III) modified diamine silver staining of DNA. The developing time was 10 min.

published a diamine silver staining procedure for silver staining of DNA in gels which had the highest sensitivity among various silver staining procedures studied (data not shown). However we found that the sensitivity of this method could be further enhanced by chromium (III) (Figure 2 and Table 2). Chromium (III) facilitates the developing of gels and improves the contrast and sensitivity of gels (Figure 2 and Table 2). Bands with more than 5 ng DNA can be directly visualized after the chromium (III) reaction steps without further developing with citric acid and formaldehyde (data not shown). The detection limit of the published diamine silver staining of dsDNA was about 100 to 500 pg per band to respective dsDNA fragments (Vari & Bell 1996). However, the detection limits of the chromium (III) modified diamine silver staining was 10 to 30 pg per band (Table 2), which was an enhancement of about 10- to 25-folds. Our new staining

Table 2. The detection limits of previous published and modified diamine silver staining of four proteins (pg per band) and six dsDNA fragments (pg per band).

Procedures	Rabbit actin	Bovine carbonic anhydrase	Trypsin inhibitor	Hen egg white lysozyme		
I	225 ± 153	600 ± 409	275 ± 137	475 ± 205		
II	28 ± 7	75 ± 41	30 ± 19	103 ± 41		
III	23 ± 8	73 ± 30	40 ± 19	125 ± 50		
IV	3 ± 1 (17)	9 ± 3 (13)	4 ± 2 (50)	83 ± 45 (6)		
VII	50 ± 10	120 ± 50	200 ± 110	480 ± 160		
Procedures	dsDNA fragments of DNA marker (bp)					
	2000	1000	750	500	200	100
V	100	100	100	100	250	500
VI	10 (10)[b]	10 (10)	10 (10)	10 (10)	10 (25)	30 (17)

Procedures from I to VI were the same as those in Table 1. Procedure VII, the published CBB-Cr (III) modified thiosulfate-silver staining of proteins (Zhou *et al*. 2002).

[a]Times of sensitivity enhancement of the procedure IV, compared with the sensitivity of procedure VII. The significance of differences of detection limits between procedures IV and VII was obvious, at least $p < 0.05$.

[b]Times of sensitivity enhancement of the procedure VI, compared with the sensitivity of the procedure V. The significance of differences of detection limits between procedures VI and V was obvious, at least $p < 0.05$. All data shown represent mean ± standard error, based on experiments of five replicates.

method might be the most sensitive procedure for silver staining of dsDNA in gels presently.

Since the sensitivity of diamine silver staining of proteins and dsDNA in gels could be further improved by the chromium (III), we propose that these sensitive silver staining procedures should be used to detect trace amounts of proteins and DNA which can not be effectively detected by previous established methods. Furthermore, it would be useful to further evaluate if chromium (III) could also be used to enhance the sensitivities of silver staining of other biomolecules, such as RNA, lipids and sialoglycoproteins in gels.

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