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The effect of sodium tetradecyl sulfate on mobility and antigen detectability of microtubule proteins in sodium dodecyl sulfate-polyacrylamide gel electrophoresis

Several factors been reported to influence the mobility of polypeptide in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) including the brand of SDS. Using microtubule proteins from axonemes of *Lytechinus pictus* and *Spisula solidissima* sperm and meiotic spindles of *Spisula solidissima* we demonstrate that the change in mobility was caused by sodium tetradecyl sulfate (STS), a major contaminant of many commercial SDS brands. We also examined the use of sodium tetradecyl sulfate and different SDS brands as a tool in extracting more information from immunoblot studies. Commercial SDS containing contaminants other than sodium tetradecyl sulfate reduced or eliminated the immunosignal from certain polypeptides and the loss of antigenicity could not even be recovered by immunoblot under "renaturing" conditions. It can thus be concluded that STS can be useful in separating and identifying comigrating polypeptides and in detecting additional immunobands in immunoblots.

1 Introduction

Sapiro *et al.* [1] initially described sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as a rapid and simple tool to estimate the size of polypeptides. Two years later Weber and Osborn [2] demonstrated the usefulness of this technique on more than 40 proteins. Since then, SDS-PAGE has developed into a standard technique in cell biology and protein biochemistry. By using SDS-PAGE, a precise molecular weight determination of polypeptides is, however, not possible; *e.g.* tubulin, a 50 kDa polypeptide, migrates as a 55 kDa polypeptide band in SDS gels. The migration of polypeptides in SDS-PAGE can be effected still further by factors such as phosphorylation, glycosylation, or unusual amino acid composition (*e.g.*, proline-rich proteins). Protein modifications are not the only factors that influence the mobility of polypeptides in SDS-PAGE. Matheka *et al.* [3] noted that certain viral proteins reveal a different apparent molecular weight depending on the brand of SDS used. They indicated that a contamination of SDS with a longer-chained alcohol might cause the change in mobility. Most commonly, sodium tetradecyl sulfate (STS) is found as a contaminant in SDS. In this paper we will describe the effect of STS on the migration of polypeptides in SDS polyacrylamide gel electrophoresis on microtubule proteins obtained from sperm axonemes and meiotic spindles of the echinoderm, *Lytechinus pictus* and the mollusc *Spisula solidissima*. Furthermore, we will discuss the possible interference of SDS brands and SDS contaminants with the recognition of antigens in SDS-PAGE immunoblots.

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2 Materials and methods

2.1 Isolation of protofilament ribbons from sperm axonemal microtubules

Protofilament ribbons were isolated from axonemal microtubules of sea urchin and surf clam sperm as described previously [4, 5]. Sperm was released by dissecting and incubating the gonad in seawater. Sperm were separated from tissue by filtering through a double layer of cheese cloth and pelleting at 1000 *g* for 5 min. Pelleted sperm were resuspended in axoneme storage buffer (10 mM Tris-HCl, pH 8.0, 150 mM KCl, 5 mM $MgSO_4$, 0.5 mM EDTA) and homogenized until about 90% of the heads and tails were detached. Sperm tails were purified by differential centrifugation. The membrane was removed by resuspending the pellected sperm tails in axoneme storage buffer containing 1% Nonidet P-40. Axonemes were washed twice with axoneme storage buffer and then fractionated into Sarkosyl-insoluble ribbons by extraction with 0.5% Sarkosyl in 50 mM Tris-HCl, pH 8.0, 1 mM EDTA, 1 mM DTT twice for 30 min. All steps were performed at 4°C. For SDS-PAGE and SDS-PAGE immunoblot, 10–15 μg protein was loaded into each gel lane.

2.2 Isolation of meiotic spindles from *Spisula* oocytes

Isolated spindles were prepared as described previously [5]. Artificially activated eggs were washed first with 1 M glycerol, 10 mM sodium phosphate, pH 7.8, and then with unbuffered 1 M glycerol. After the second wash, pelleted eggs were resuspended in lysis buffer, containing 10 mM piperazine-*N,N'*-bis(2-ethanesulfonic acid), pH 6.6, 5 mM EGTA, 1 mM $MgCl_2$, 20% glycerol, 1% Nonidet P-40. To obtain clean spindle preparations, spindles were filtered through a 48 μm nylon filter and washed twice with lysis buffer without detergent.

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2.3 SDS-PAGE

SDS-PAGE was conducted according to Laemmli [6] using a Hoefer Mighty Small II slab gel apparatus (San Francisco, CA). A Hoefer SE400 vertical slab gel unit was employed for high resolution protein separation. Briefly, 7.5% w/v acrylamide gels were prepared from a stock solution containing 30% w/v acrylamide and 0.8% w/v Bis-acrylamide leaving out the SDS. A 3% w/v acrylamide gel was used to stack the protein samples. All gels were prepared without SDS. Pure SDS or an SDS/STS mixture of up to a total concentration of 0.1% w/v was added later to the running buffer (for more details see Section 3.2). Samples were dissolved in Laemmli sample buffer in the presence of 2% w/v ultrapure SDS from Bio-Rad (98%, w/w pure, Richmond, CA), while incubating at 100°C for 5 min. Proteins were either stained with 0.0175% w/v Serva Blue in 25% v/v isopropanol, 10% v/v acetic acid or they were transferred to nitrocellulose to be used for immunoblotting.

2.4 Immunoblotting

Two different transblot procedure were employed: (i) electrotransblot according to Towbin *et al.* [7] in the presence of SDS or (ii) electrotransblot under 'renaturing' condition according to Dunn [8]. Using Towbin's procedure, proteins were electrotransferred in transblot buffer containing 25 mM Tris, 192 mM glycine, 20% v/v methanol, and 0.1% w/v SDS (pH 8.8, nonadjusted) at 60 V for 2 h. To remove the SDS, transfer was continued for an additional 30 min in transblot buffer lacking SDS. Using Dunn's procedure, gels were presoaked in 20% w/v glycerol, 50 mM Tris, pH 7.4 for 1 h. Proteins were then electrotransferred in a buffer containing 10 mM $NaHCO_3$, 3 mM Na_2CO_3, 20% v/v methanol, pH ~9.9 (nonadjusted) at 60 V for 2.5 h. After transferring the proteins, nitrocellulose sheets were rinsed several times with distilled H_2O, stained with Ponceau S for 10–30 s, and then destained with distilled H_2O. Fifty mL Ponceau S solution was prepared by first dissolving 10 mg Ponceau S in 1.5 mL 100% w/v solution of trichloroacetic acid (Sigma, St. Louis, MO). After taking photographs of the nitrocellulose sheets, the Ponceau staining was removed in additional washes with distilled H_2O. Fast removal of the stain was achieved by adding a drop of 1 N NaOH to the distilled H_2O and rocking it for about 1 min. Nitrocellulose was rinsed several times with 20 mM Tris-HCl, 500 mM NaCl, pH 7.5 (TBS) and blocked with 3% w/v BSA in TBS for 20 min. Alternatively, nitrocellulose sheets were blocked with Tween 20 by incubating them first in PBS containing 0.3% w/v Tween 20 for 30 min at 37°C, followed by three 15 min incubations in PBS-Tween at room temperature. Tektins were identified using affinity-purified, polyclonal antibodies raised against tektins from sea urchin sperm [9] at a concentration of 1–10 μg/mL. Immunoblotting was conducted as follows: replicas of single lanes were incubated with a primary antibody in TBS containing 1% w/v BSA for 60 min at room temperature, washed three times for 10 min each with TBS, incubated with secondary antibody, and finally washed three times, 10 min each with TBS. As secondary antibody, a peroxidase-conjugated goat anti-rabbit (Bio-Rad) was used at a dilution of 1:2000 in 1% w/v BSA, TBS; 4-chloro-1-naphthol or luminol/luciferin [10] was used as detection system for the peroxidase.

3 Results and discussion

3.1 Composition of axonemal ribbons of sperm from sea urchin and surf clam

Best *et al.* [11] have indicated that the separation of α- and β-tubulin is influenced by the composition of commercial SDS brands. The wall of axonemal microtubules consists of tubulin and of additional structural components, called tektins. Tektins were initially described by extracting axonemal microtubules of sea urchin sperm, first with sarkosyl and then with sarkosyl/urea [12]. The sarkosyl-resistant structure consists of ribbons of three protofilaments, while the sarkosyl/urea insoluble material consists of filaments 2–6 nm in diameter. Figure 1 compares sarkosyl-resistant protofilament ribbons from the sea urchin *Lytechinus pictus* (lane 2) and the surf clam *Spisula solidissima* (lane 1). The overall composition of the protofilament ribbons appeared quite similar: α- and β-tubulin, a pair of polypeptides of ~77 and ~ 83 kDa and several polypeptides below tubulin. In *L. pictus* three tektins had been

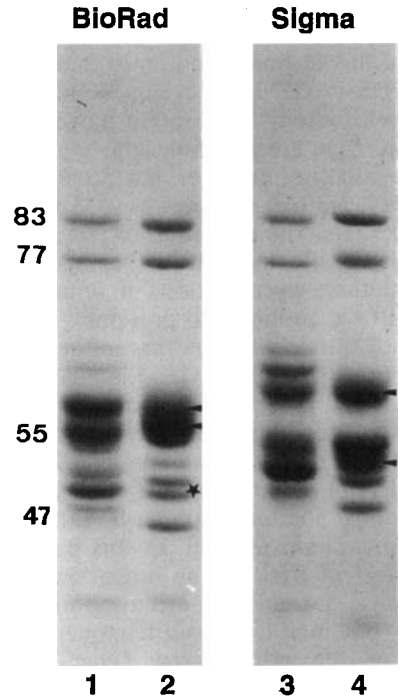

Figure 1. SDS-PAGE of sarkosyl-resistant protofilaments of axonemal microtubules separated in the presence of two different brands of SDS. Lanes (1) and (3), protofilaments from *Spisula* sperm. (2), (4) Protofilaments from *Lytechinus* sperm. Protofilament ribbons were separated either in the presence of 0.1% w/v SDS from Bio-Rad (1) and (2) or in the presence of 0.1% w/v SDS from Sigma (3) and (4) in a 12 cm analytical, 7.5% w/v polyacrylamide gel. The purity of the Bio-Rad SDS was 98% w/w, while the Sigma SDS contained only 67% w/w sodium dodecyl sulfate. Arrowheads indicate the position of α- and β-tubulin. * indicates the position of the tektin B doublet band in axonemal ribbons of *Lytechinus pictus*. The identity of the proteins had already been confirmed by 2-D IEF/SDS-PAGE [9] and immunoblot [13]. Numbers at the left-hand side indicate the apparent molecular mass in kDa.

identified with an apparent molecular mass of about 47 kDa for tektin C, of about 51 kDa for tektins B, and of 55 kDa for tektin A [12]. By analytical SDS-PAGE (12 cm running gel) using ultrapure SDS (98% w/w pure) from Bio-Rad, tektin B of *L. pictus* separated into two polypeptide bands, while α- and β-tubulin were hardly separated (Fig. 1, lane 2). In *Spisula solidissima* the mobility of tektins differed from that of *L. pictus* and the three tektins could not be identified by SDS-PAGE (compare Fig. 1, lane 1 and 2).

The migration of microtubular proteins was substantially altered when SDS from Sigma was employed in the gel system. The Sigma SDS contained 67% w/w sodium dodecyl sulfate (SDS), 26% w/w sodium tetradecyl sulfate (STS) and 6% w/w sodium hexadecyl sulfate (SHS). The change in mobility of the microtubular proteins from *L. pictus* sperm is summarized in Table 1. By switching from the ultra pure Bio-Rad SDS (98% w/w pure) to Sigma SDS (67% w/w pure) a major change was observed in the mobility of α- and β-tubulin. Whilst α- and β-tubulin were separated by about 2 kDa and the Bio-Rad SDS-PAGE, a separation of about 7 kDa was noted in the Sigma SDS-PAGE (Fig. 1, lanes 2 and 4 and Table 1). Furthermore, the relative position of sea urchin β-tubulin and tektin A was reversed, an observation already made by Stephens [13, 14]. A very similar observation was made in terms of the mobility of α- and β-tubulin from *Spisula* (Fig. 1, lanes, 1 and 3). Using a 0.5% sarkosyl/2 M urea extraction, α- and β-tubulin could be removed completely, leaving behind the insoluble tektin filaments, some of which had an SDS-PAGE mobility between α- and β-tubulin (data not shown).

3.2 Effect of tetradecyl sulfate on the mobility of axonemal proteins

Matheka *et al.* [3] proposed that a contamination of some SDS brands with longer-chained alcohol is responsible for the altered mobility of certain polypeptides by SDS-PAGE. Our results obtained from the comparison of Bio-Rad SDS (98% w/w pure) and Sigma SDS (67% w/w pure) support this assumption. To test this hypothesis, ultra pure SDS from Bio-Rad (98% w/w) or Pierce (99% w/w; Rockford, IL) was gradually supplemented with increasing amounts of STS in the running buffer. The total concentration of SDS/STS used in the running buffer was always 0.1% w/v. The running buffer was altered by replacing part of the Bio-Rad SDS with STS from a 10:0 ratio for SDS:STS (Fig. 2, lane 1 and 1′) to a 7:3 ratio for SDS:STS (Fig. 2, lane 8 and 8′). With increasing concentration of STS a wider separation of α- and β-tubulin was observed. Furthermore, at the highest concentration of STS a distinct polypeptide band migrated between α- and β-tubulin (arrowheads in Fig. 2); this was also the case when Sigma SDS was used (compare with Fig. 1). According to observations made by Stephens [13] this polypeptide band most likely represents tektin A. Most of the other polypeptides revealed only a minor alteration in mobility. Using STS to supplement the SDS in this way during protein separation, the longer chain alcohol STS can indeed change the mobility of certain polypeptides.

3.3 Effect of STS on immunoblot

As demonstrated above, STS affected the SDS-PAGE mobility of certain microtubule proteins. To identify polypeptides within a protein mixture, SDS-PAGE immunoblot provides a useful method; however, the method has its limitations for comigrating polypeptides. Here, we utilized the ability of STS to affect polypeptide mobility in order to gain information on functional relationships between polypeptides from SDS-PAGE immunoblot. SDS-PAGE immunoblot was carried out either in the presence of Bio-Rad SDS, Sigma SDS or Bio-Rad SDS

Table 1. Apparent molecular mass of microtubule proteins of axonemes from *Lytechinus pictus* in the presence of Bio-Rad SDS and Sigma SDS

	Bio-Rad SDS	Sigma SDS
	Molecular mass (kDa)	
Component-83	83	84
Component-77	77	77
α-Tubulin	58	59
β-Tubulin	56	52
Tektin A	55	53
Tektin B	51	51
Tektin C	47	47.5

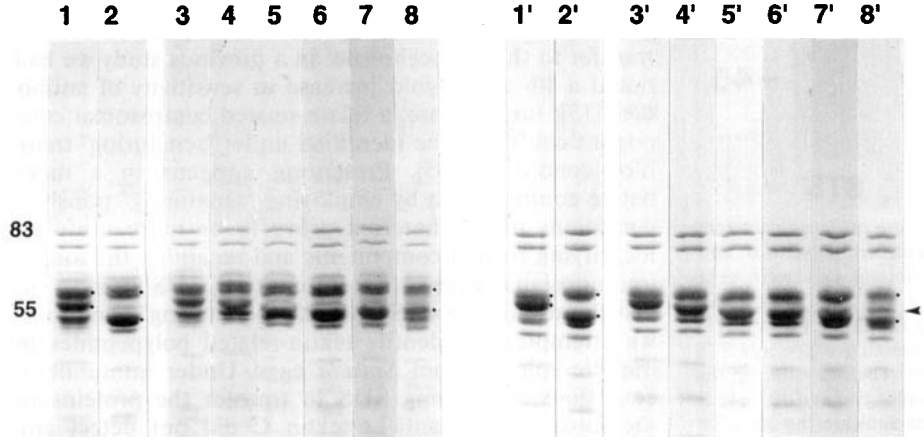

Figure 2. SDS-PAGE of sarkosyl-resistant protofilament ribbons in the presence of increasing concentrations of STS in the running buffer. Proteins from *Spisula solidissima* (lanes 1–8) and *Lytechinus pictus* (lanes 1′–8′) were dissolved in a Bio-Rad-containing sample buffer and then separated by SDS-PAGE by replacing part of the ultra pure Bio-Rad SDS of the running buffer with increasing amounts of STS; 0.1% SDS/0% STS lanes 1 and 1′), 0.095% SDS/0.005% STS (3), (3′), 0.09% SDS/0.01% STS (4), (4′), 0.085% SDS/0.015% STS (5), (5′), 0.08% SDS/0.02% STS (6), (6′), 0.075% SDS/0.025% STS (7), (7′), and 0.07% SDS/0.03% STS (8), (8′). SDS-PAGE was performed in a Hoefer Mighty Small gel apparatus containing 7.5% polyacrylamide. For comparison, lanes (1) and (2) demonstrate SDS-PAGE in the presence of Bio-Rad SDS and Sigma SDS, respectively. Dots indicate the position of α- and β-tubulin. Arrowhead indicates the position of tektin A. Numbers at the left-hand side indicate the apparent molecular mass in kDa.

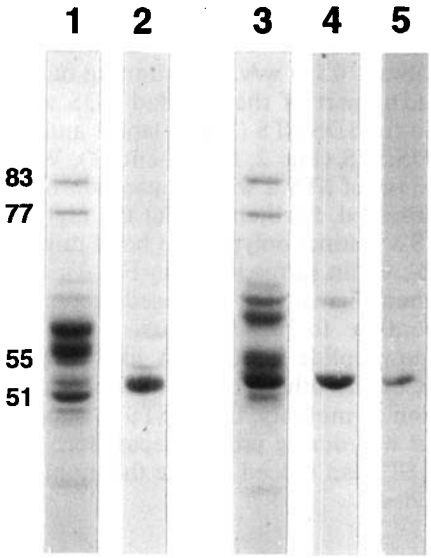

Figure 3. SDS-PAGE immunoblot using different electro-transfer conditions. Lanes (1) and (2), SDS-PAGE of axonemal ribbons from *Spisula* sperm separated in the presence of Bio-Rad SDS: (1) Serva Blue-stained gel; (2) immunoblot stained with anti-Lp-tektin B. (3), (4) SDS-PAGE of axonemal ribbons from *Spisula* sperm in the presence of Bio-Rad SDS suplemented with 0.03% STS: (3) Serva-Blue-stained gel; (4) immunoblot stained with anti-Lp-tektin B. (5) SDS-PAGE immunoblot in the presence of Sigma SDS and labeled with anti-Lp-tektin B. Numbers at the left-hand side indicate the apparent molecular mass in kDa.

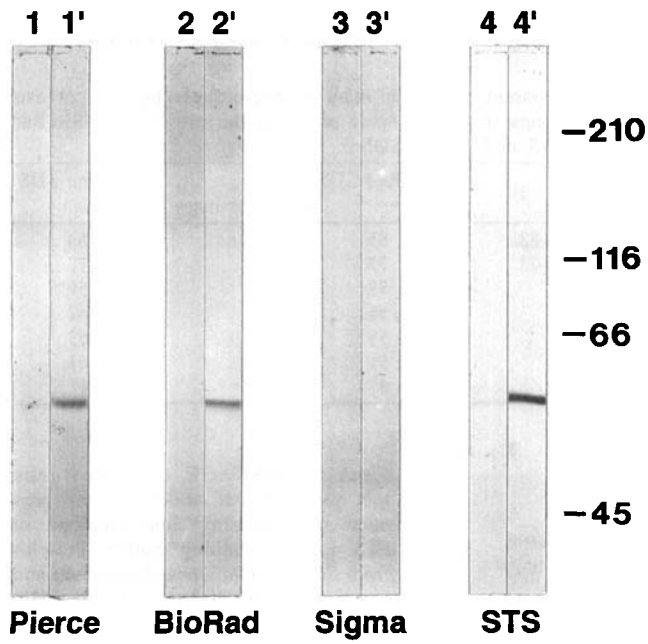

Figure 4. Effect of SDS and STS on the detection of *Spisula* antigens by SDS-PAGE immunoblot using affinity-purified polyclonal anti-Lp-tektin C. Polypeptides of spindles from *Spisula* eggs were separated by SDS-PAGE in the presence of 0.1% w/v SDS from Pierce (lanes 1 and 1'), 0.1% w/v SDS from Bio-Rad (2), (2'), 0.1% w/v SDS from Sigma, and 0.07% w/v SDS (Pierce) containing 0.03% w/v STS (4), (4'). Proteins were then transferred to nitrocellulose either in the presence of the respective SDS (1)–(4) or under "renaturing" condition (1')–(4'). Numbers at the right-hand side indicate the apparent molecules mass in kDa.

supplemented with STS (Figs. 3 and 4). The polyclonal antibody, anti-Lp-tektin B, is monospecific for tektin B, an ~51 kDa component in sea urchin axonemes [9]. One of the standard techniques calls for the presence of SDS in the transfer buffer to improve the mobility out of the gel onto the nitrocellulose. When sarkosyl-resistant ribbons of *Spisula* sperm were probed with anti-Lp-tektin B, the antibody reacted strongly with a 52 kDa component (Fig. 3, lane 2). It is thus likely that the 52 kDa represents tektin B in *Spisula solidissima*. The mobility of the 52 kDa immunoband was not altered when Sigma SDS or Bio-Rad SDS supplemented with STS were used. However, the intensity of the immunosignal was substantially reduced in the presence of Sigma SDS (Fig. 3, lane 5). In addition to the labeling of the 52 kDa polypeptide, anti-Lp-tektin B weakly cross-reacted with an ~54 kDa component which migrated just below β-tubulin (Fig. 3, lane 1 and 2). In the presence of STS during SDS-PAGE and electro-transfer an ~61 kDa instead of the 54 kDa component was detected (Fig. 3, lane 4) suggesting that the 54 kDa component had altered its mobility and was now clearly separated from α-tubulin. In contrast, the minor 61/54 kDa polypeptide was not detectable in SDS-PAGE immunoblot using Sigma SDS (Fig. 3, lane 5). The cross-reaction of the tektin B-specific antibody could indicate that the 61/54 kDa polypeptide might represent another tektin functionally related to tektin B; however, a definite identification of the 54/61 kDa component will only be possible by 2-D IEF/SDS-PAGE immunoblot.

It is known that SDS can interfere with the sensitivity of antibodies by SDS-PAGE immunoblot. In such cases, the interference may either be due to a binding of SDS to the epitope or due to a destruction of the epitope during SDS-PAGE. As demonstrated above, similar polypeptide patterns were obtained with Sigma SDS and Bio-Rad SDS, which had been supplemented with STS (Fig. 2). However, the use of Sigma SDS resulted in a reduction or loss of immunosignal (Fig. 3) which was not seen in the presence of added STS. This result indicates that contaminants of commercial SDS brands other than STS might be responsible for the loss of immunosignal.

Dunn [8] has described a method to transfer the proteins to the nitrocellulose under "renaturing" conditions. These conditions involve removal of the SDS by incubating the gel in a glycerol-containing Tris buffer prior to electro-transfer to the nitrocellulose. In a previous study we had noted a 10- to 100-fold increase in sensitivity of antibodies [15]; furthermore, a tektin-related centrosomal component could only be identified under "renaturing" transblot conditions [15]. Presenting antigens in a more native conformation by employing "renaturing" transblot conditions might therefore prove to be more useful in identifying related components and regaining the loss of immunosignal caused by commercial SDS brands. To test the usefulness of STS under "renaturing" conditions we attempted to identify tektin-related polypeptides in meiotic spindle from *Spisula* eggs. Under immunoblot conditions, employing SDS to transfer the proteins to the nitrocellulose anti-Lp-tektin C did not detect any component in meiotic spindles. The negative result was independent of the brand of SDS used (Fig. 4, lanes 1–4). However, the affinity-purified polyclonal antibody raised against tektin C, a 47 kDa component in sea

urchin sperm, detected a 52 kDa component in SDS-PAGE replicas under "renaturing" conditions ([5] and Fig. 4, lanes 1′ and 2′). The 52 kDa component was missing when SDS from Sigma (Fig. 4, lane 3′) was used during electrophoresis and it was present when Bio-Rad SDS was supplemented with STS (Fig. 4, lanes 4′), again indicating that contaminants of commercial SDS other than STS interfered with the immunoblot. The mobility of the 52 kDa spindle component and the 47 kDa axonemal tektin (not shown) was not influenced by different SDS-PAGE conditions. Based on the difference in mobility, the 52 kDa component might represent a spindle-specific tektin distinct from the axonemal tektin C [5].

4 Concluding remarks

In the present study we demonstrate that the mobility shift of the proteins previously observed with certain commercial SDS brands is caused by sodium tetradecyl sulfate, a major contaminant of commercial SDS. SDS-PAGE has its limitations in resolving comigrating polypeptides; we show that adding STS during SDS-PAGE could provide an alternative to 2-D IEF/SDS-PAGE in separating comigrating polypeptides such as microtubule proteins. By SDS-PAGE immunoblot, the appropriate epitopes can frequently be masked or destroyed by the presence of SDS. In the studies of Dunn [8] and our own [15] it was demonstrated that many antigens could be recovered by the use of "renaturing" conditions. Our data indicate that in the presence of certain SDS contaminants other than STS immunosignals cannot be recovered under "renaturing" conditions, suggesting that these non-STS contaminants either destroy or prevent re-folding of the polypeptides. In summary, the SDS brands can have a profound effect on results obtained and the use of STS and various SDS brands in gel electrophoresis and immunoblot can provide a useful tool in characterizing related polypeptides of similar molecular weight.

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