Separation of Low Molecular Weight Proteins with SDS-PAGE Using Taurine as a New Trailing Ion

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Key words: SDS-PAGE — Ca^{2+}-binding Proteins — Peptides — Tricine — Taurine

Abstract. Taurine as an alternative trailing ion for tricine yields an identical resolution but reduces the running time by 15% and the power consumption by 15% compared to tricine. Therefore cooling of gels is more effective in SDS PAGE with taurine and artefacts due to oxidation of proteins can thus be reduced.

Introduction

More than 200 Ca^{2+} binding proteins (CaBP) are known to either act as Ca^{2+} buffers or as Ca^{2+}sensors transducing the ubiquitous Ca^{2+}signal into cell specific responses by binding to specific target proteins (Heizmann and Hunziker 1991). Since the differential expression of CaBP like the S100 family is associated with various diseases (Schäfer and Heizmann 1996) there is growing interest in expression analyses involving SDS PAGE and immunoblotting techniques that allow a high resolution of these small acidic proteins with a molecular weight between 8 and 22 kDa. The well-established and highly reproducible method of protein electrophoresis as published by Laemmli et al. (Laemmli 1970) gives an excellent resolution for proteins between 20,000 and 200,000 Da. Proteins below 10,000 Da, however, show a similar size as SDS (sodium dodecyl sulphate) micelles (Fish et al. 1970) and thus migrate at the SDS front-line. Although the introduction of tricine instead of glycine as trailing ion by Schägger and von Jagow (Schägger and von Jagow 1987) has greatly improved the resolution of small proteins, artefacts are often seen that generally are due to an increased heat generation when a high acrylamide crosslinking is used. In this respect the oxidation of proteins by acrylamide

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monomers (Bonaventura et al. 1994) and the development of multiple bands by disulfide crosslinking of proteins (Kumar et al. 1993) have been discussed.

Here we describe the use of taurine as a new trailing ion that is less costly and shows a comparable resolution of small proteins as tricine but reduces both running time and power consumption due to a more effective electric field generation.

Materials and Methods

Taurine (2-Aminoethanesulfonic acid) and tricine (N-tris-(Hydroxymethyl)-methyl-glycine-N-(2-Hydroxy-1,1-bis[hydroxymethyl]-ethyl)-glycine) were purchased from Sigma (Munich, Germany). Mark12^{TM} protein standard was from Novex (San Diego, CA, U.S.A). All other chemicals were obtained from Merck (Darmstadt, Germany) Electrophoresis gels were run in a vertical Hoefer chamber (Pharmacia, Freiburg, Germany). Plates were cooled by the anode buffer, whose temperature was set to a constant +15°C. Gel dimensions were 12 × 14 × 0.1 cm (length ×

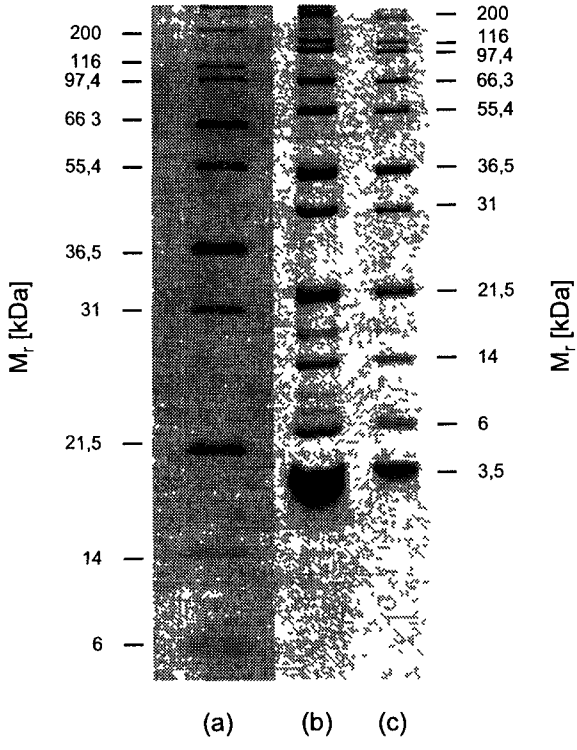

Figure 1. Representative results for separation of Novex Mark12 protein standard by SDS-PAGE (15% T, 3% C) using several buffer systems (a) Lammli (Tris/glycine), (b) Schagger and von Jagow (Tris/tricine), (c) Tris/taurine Molecular weights of the standard proteins are given in kDa Silver stain

width × depth) for separating gels and 2 × 14 × 0.1 cm for stacking gels. According to the notation of Hjerten (Hjerten et al. 1965) for comparison of different buffer systems we used acrylamide concentrations of 15% T (Total) and 3% C (Crosslinker) for separating gels and 5% T and 3% C for stacking gels. Gels were prepared from a 48.5% acrylamide, 1.5% bisacrylamide stock containing 0.5 M Tris-HCl pH 8.6, 0.1% TEMED (N,N,N′,N′-Tetramethylethylenediamine, Sigma, Munich, Germany). Polymerisation was started by the addition of 100 μl of a 10% ammonium persulfate (APS) stock per 10 ml gel preparation. Electrophoresis buffers contained 100 mM Tris, 0.1% SDS, and either 100 mM taurine or 100 mM tricine. The Laemmli buffer consisted of 25 mM glycine, 70 mM Tris, 0.1 % SDS. Gels were run at a constant current of 30 mA/cm^2 and stopped shortly before the bromphenolblue band had reached the lower edge of the gel. Gels were silver stained according to Oakley (Oakley et al. 1980). R_f 1.0 was set at the position of the bromphenolblue band.

Results and Discussion

Both the silver stained SDS PAGE and the relative mobility plotted against the M_r demonstrate that in our experimental setting taurine and tricine show a similarly increased protein resolution capacity as compared to glycine (Fig. 1 and 2). Figure 3 depicts the power plotted against running time needed for protein resolution as shown in Figure 1. Interestingly taurine trailing resulted in a reduction of running time and power consumption by 15% compared to tricine trailing.

Consequently, a lower heat generation means a separation at lower temperature and less frequent heat associated denaturing of proteins as described above.

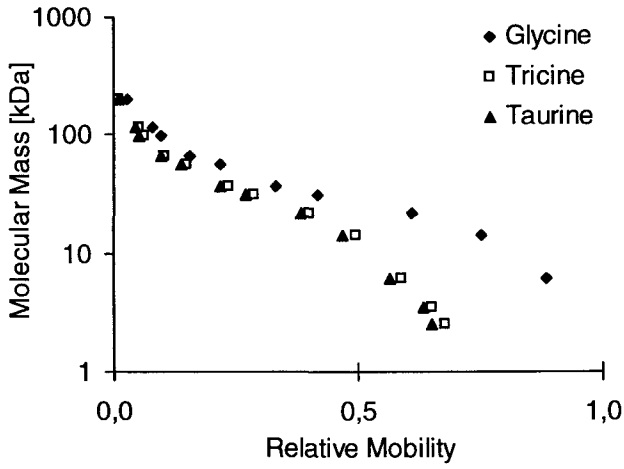

Figure 2. Calibration curves of protein resolution achieved with the three buffer systems (see Figure 1).

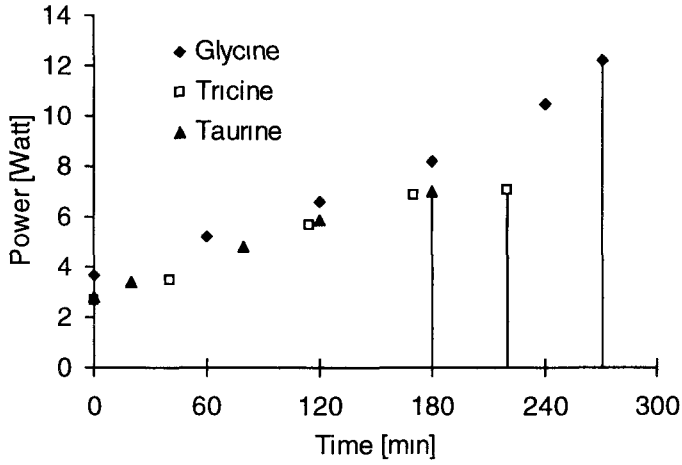

Figure 3. Power consumption of the three buffer systems Electrophoresis with Tris/taurine was stopped after 180 minutes, with Tris/tricine after 220 minutes, and with Tris/glycine after 270 minutes (vertical lines)

This again is associated with an improved protein transfer to PVDF (polyvinylidene difluoride) or nitrocellulose membranes. Taurine trailing might thus improve sensitivity of Western blotting of small hydrophobic CaBP that otherwise poorly migrate from gel to membrane in an electrical field (Frank et al. 1993). The higher resolution capacity and improved running conditions should also allow an improved detection of proteins with a higher M_r and very low abundance since lower acrylamide concentrations can be employed in these cases Additionally the lower price for taurine compared to tricine implies a significant cost reduction when SDS PAGE is part of laboratory routine.

The taurine system was highly efficient in separating several isoforms of S100 proteins which have a molecular weight between 8 and 13 kDa Thus it is the method of choice for quality control of purification procedures of these proteins (Ehlermann et al. 2000).

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Final version accepted May 10, 2001

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