Protein purification for the Lowry assay: acid precipitation of proteins in the presence of sodium dodecyl sulfate and other biological detergents.

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Analytical Biochemistry 1998;265(2):381-4

Abstract

The Lowry protein assay is widely used to quantify proteins in the laboratory. As the assay is susceptible various interfering substances, the protein test sample is frequently first purified by precipitating the proteins using trichloroacetic acid (TCA). However, TCA precipitates proteins poorly in the presence of sodium dodecyl sulfate (SDS), a detergent very commonly used in biological research. The inhibitory effect of up to 1% SDS on protein precipitation is effectively overcome by a combination of 5% TCA and 1% phosphotungstic acid (PTA). The TCA/PTA mixture also precipitates proteins readily in the presence of other detergents such as 0.1% Tween 20, Triton X-100 or Nonidet P-40. TCA/PTA has the capacity to precipitate a wider cross-section of proteins than TCA alone, the combination of acids remaining effective in the presence of SDS and other detergents.

NOTES & TIPS

Protein Purification for the Lowry Assay: Acid Precipitation of Proteins in the Presence of Sodium Dodecyl Sulfate and Other Biological Detergents[1]

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Received May 20, 1998

As the Lowry protein reaction (1) is susceptible to interference by various substances (2), purification of the protein sample by precipitation with acids prior to protein assay is frequently undertaken (1, 3, 4). Among the interfering substances are various biological detergents (2). Such interference can be overcome by the addition of sodium dodecyl sulfate (SDS) when carrying out the Lowry assay (5, 6). Notwithstanding this, SDS, a commonly used detergent in biological research, may already be present in some test samples prior to acid precipitation. How SDS and other detergents in the test sample affect the acid precipitation step to purify proteins (rather than the Lowry reaction itself) has not been investigated. This report describes the effect of SDS on protein purification by precipitation with trichloroacetic acid (TCA)[3] and posphotungstic acid (PTA) to prepare for the micro Lowry protein assay. The effects of some other biological detergents (Tween 20, Triton X-100, and Nonidet P-40) on acid precipitation of proteins are also examined. Ovalbumin was used as the test protein in initial experiments while further investigations involved proteins eluted from natural rubber latex gloves.

[1] This work was supported by IRPA Grant 01-04-04-0007 from the Ministry of Science, Technology, and the Environment, Malaysia.

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[3] Abbreviations used: TCA, trichloroacetic acid; PTA, phosphotungstic acid; PBS, phosphate-buffered saline.

ANALYTICAL BIOCHEMISTRY 265, 381–384 (1998)
ARTICLE NO. AB982893
0003-2697/98 $25.00

Materials and Methods

Reagents. Ovalbumin, TCA, PTA, SDS, Tween 20 (polyoxyethylene sorbitan monolaurate), Triton X-100, Nonidet P-40, and Folin–Ciocalteu reagent were purchased from Sigma Chemical Co., U.S.A. Reagents for the modified micro Lowry assay were prepared as follows: Reagent A, 6% sodium carbonate in distilled water; Reagent B, 1.5% copper sulfate in 3% sodium citrate; Reagent C, 50 ml Reagent A mixed with 1 ml Reagent B (working reagent prepared on the day of assay); and Reagent D, Folin reagent diluted to 72% with distilled water.

Concentrations of all reagents and test proteins refer to their final concentrations in the mixture.

Test proteins. Ovalbumin (90 μg ml ^{1}) was dissolved in distilled water or in distilled water containing 0.1 or 1% SDS or 0.1% of the detergent Tween 20, Triton X-100, or Nonidet P-40.

Pieces of latex gloves (equal weight from five brands) were eluted in phosphate-buffered saline (PBS) at the rate of 290 g per 500 ml buffer for 3 h. A portion of the eluate was dialyzed overnight at 4°C in 2000-kDa molecular weight cut-off dialysis tubing (Sigma Chemical Co.) against PBS. Dialysis removed most of the substances that would interfere with the Lowry assay (3). The dialyzed sample was used for the Lowry reaction without acid precipitation, while the Lowry assay was carried out on the undialyzed sample after acid precipitation with TCA or a mixture of TCA and PTA.

Protein purification and concentration by acid precipitation. In a typical assay, an aliquot of 0.5 ml containing 35% (w/v) TCA and 7% (w/v) PTA was added to 3 ml of test sample (or other volumes in a similar ratio) in a centrifuge tube. After mixing, the mixture was allowed to stand for 20 min. Centrifugation was then carried out at 2060*g*, 4–6°C, for 30 min on a Beckman GS-6R centrifuge, after which the supernatant in the centrifuge tube was discarded. The centrifuge tube was left in an inverted position for several minutes to ensure thorough removal of the supernatant. Plastic (polycarbonate) centrifuge tubes were preferred to glass tubes as the former retained the precipitate better (in the form of a thin film at the bottom of the tube). The protein precipitate was redissolved in 3 ml 0.25 M NaOH for at least 20 min. Protein samples without detergent or that contained SDS dissolved readily in NaOH, but samples that contained the other

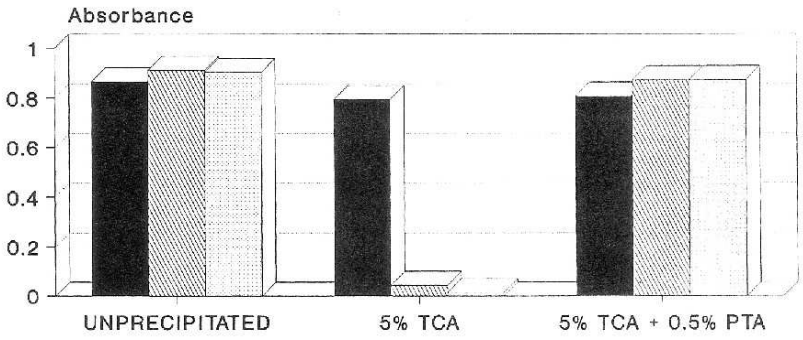

FIG. 1. Effect of 5% TCA or 5% TCA + 1% PTA in precipitating a 90 μg ml^{-1} ovalbumin sample containing no SDS ■, 0.1% SDS ▨, or 1% SDS ▩ (means of two readings). The unprecipitated samples were dialyzed.

detergents were generally more difficult to redissolve, and thorough mixing for about 30 min with intermittent vortexing was required.

Micro Lowry assay. The micro Lowry assay modified for high sensitivity (4) was adapted for this study. An aliquot of 0.3 ml Reagent C was added to 0.8 ml of test sample. After mixing, the mixture was allowed to stand for 15 min and 0.1 ml Reagent D was then added. In assays where Tween 20, Triton X-100, or Nonidet P-40 was present, 0.3 ml of 20% SDS was first pipetted into the test mixture before the addition of Reagent D to prevent the formation of a precipitate (5, 6). This step was omitted when SDS was the detergent present in the test sample. The contents of each tube were well mixed (using a vortex mixer) and Reagent D was added immediately. The mixture was allowed to stand for 30 min at room temperature and absorbance was read at 750 nm on a spectrophotometer.

Results and Discussion

SDS and other biological detergents are commonly used in laboratory manipulations and are therefore frequently present in low concentrations in samples for protein quantitation. When ovalbumin was precipitated with 5% TCA, the presence of SDS interfered with the precipitation reaction. SDS present at 0.1% greatly inhibited protein precipitation, while almost total inhibition occurred with 1% SDS (Fig. 1). However, when 5% TCA was supplemented with 1% PTA, protein was effectively precipitated (Fig. 1).

The TCA/PTA mixture also precipitated ovalbumin readily in the presence of 0.1% Tween 20, Triton X-100, or Nonidet P-40 (Fig. 2). In all cases, assay results gave readings within 10% of the unprecipitated controls (with no detergent added) using the Lowry assay. However, as these three detergents (unlike SDS) did not interfere with the precipitation of ovalbumin by TCA, the additional presence of PTA did not confer any advantage over using TCA alone.

SDS at 0.1 or 1% did not give a reading over the blank reading for the Lowry assay when precipitated with TCA/PTA. The other detergents at 0.1% gave a small absorbance of about 0.03 (equivalent to about 2 μg ml^{-1} ovalbumin). Whereas ovalbumin with SDS

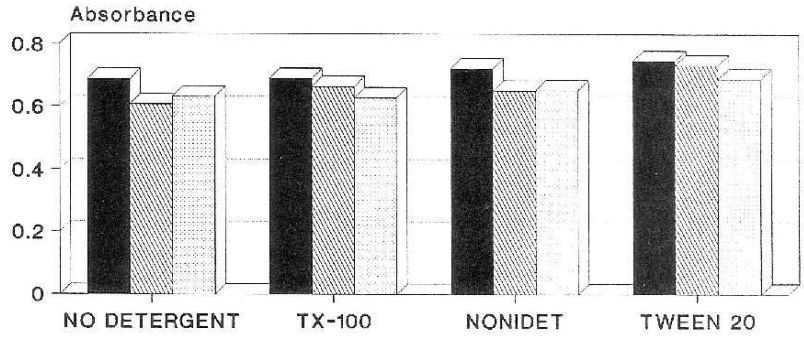

FIG. 2. Effect of various detergents (0.1%) on the quantitation of a 90 μg ml^{-1} ovalbumin sample that was unprecipitated (dialyzed) ■, precipitated with 5% TCA ▨, or precipitated with 5% TCA + 1% PTA ▩ (means of four readings). Tx-100, Triton X-100.

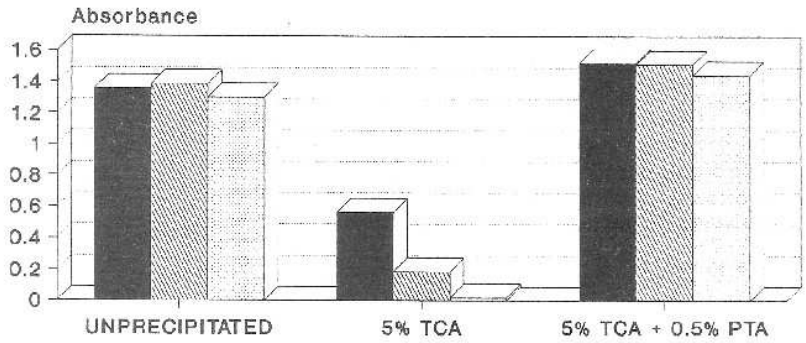

FIG. 3. Effect of 5% TCA or 5% TCA + 1% PTA in precipitating a latex glove protein sample containing no SDS ■, 0.1% SDS ▨, or 1% SDS ▩. The unprecipitated samples were dialyzed (means of two readings).

dissolved readily in NaOH, the presence of 0.1% of these other detergents made redissolving the protein difficult. For this reason, Tween 20, Triton X-100, and Nonidet P-40 were not tested at the higher concentration of 1%. Similarly, the practice of redissolving the precipitated protein in a volume of NaOH smaller than the original volume of the test sample in order to increase assay sensitivity (3, 4) was not done in this series of experiments as it would increase the detergent concentration also.

The findings obtained using ovalbumin as a protein model were compared with results obtained using proteins eluted from latex examination gloves. Latex glove proteins are a mixture of several peptides derived from *Hevea brasiliensis* latex that is used in the manufacture of surgical and examination gloves (7). Healthcare workers use latex gloves in very large numbers, and the quantitation of the very small amounts of residual proteins on these gloves is of interest in view of the important prevailing problem of latex allergy (8, 9).

Unlike ovalbumin, various latex proteins were inherently resistant to precipitation by TCA, irrespective of the presence or absence of detergents (Fig. 3). Hence, precipitating latex proteins with TCA for the purpose of protein quantitation would result in a substantial underestimate of the true protein concentration. The poor recovery of latex proteins by TCA precipitation was exacerbated by the presence of SDS (Fig. 3). When PTA was supplemented to TCA, however, the latex glove proteins were found to precipitate effectively, this action being maintained even in the presence of 0.1 or 1% SDS (Fig. 3).

Protein recovery by TCA/PTA precipitation was similarly effective in the presence of 0.1% Tween 20, Triton X-100, or Nonidet P-40. Unlike the precipitation of ovalbumin where the presence of PTA conferred no advantage over using TCA alone (Fig. 2), the added presence of PTA was clearly advantageous when precipitating latex proteins. The latex proteins that could not be precipitated using TCA alone were recovered when PTA was supplemented (Fig. 4). PTA concentration was increased from the 0.2% used previously (4) to 1% in the present study to accommodate the presence of the detergents in the test sample. If detergents are

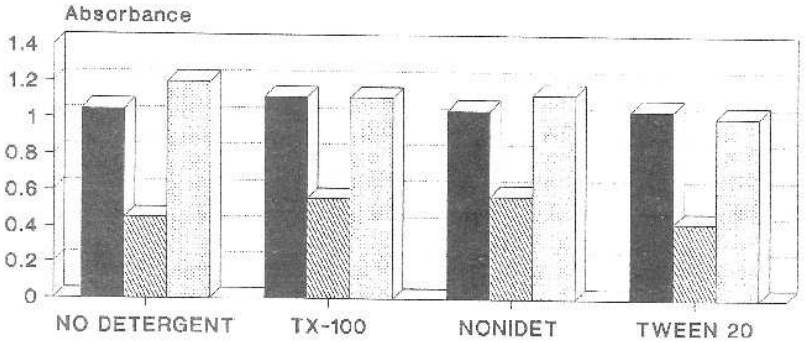

FIG. 4. Effect of various detergents (0.1%) on the quantitation of a sample of latex glove proteins that was unprecipitated (dialyzed) ■, precipitated with 5% TCA ▨, or precipitated with 5% TCA – 1% PTA ▩ (means of four readings). Tx-100, Triton X-100.

known to be absent, the use of either 0.2 or 1% PTA would be satisfactory. Wang and Smith (5) and Cadman *et al.* (6) reported that protein treated with detergent often caused a precipitate to form when the Folin reagent was added during the Lowry assay. Purification of the protein with TCA and PTA in the present study did not prevent this. Hence, the practice (5, 6) of adding SDS prior to the Folin reagent was adopted when detergents were present in the test sample. Addition of SDS in this step dilutes the reaction mixture and reduces test sensitivity slightly. Absorbance readings are higher where this step can be omitted, either where detergent is absent or where the detergent present is SDS itself (compare absorbance values in Figs. 1 and 3 with corresponding values in Figs. 2 and 4 where this step was omitted).

The tolerance limits for detergents (1% for SDS and 0.1% for Tween 20, Triton X-100, and Nonidet P-40) in protein samples apply specifically to the microassay (4). This assay has been modified for high sensitivity by increasing the proportion of the test sample in the Lowry reaction mixture. Thus, the test sample makes up 57% of the reaction mixture volume (prior to adding the Folin reagent). Higher detergent concentration can be tolerated in a typical "standard" Lowry assay where the test sample might be no more than about 5–10% of the total volume.

Conclusion

In purifying proteins by acid precipitation, 5% TCA precipitates proteins (exemplified by ovalbumin and latex proteins) poorly in the presence of SDS. However, the inhibitory effect of SDS on protein precipitation is effectively overcome by a combination of 5% TCA and 1% PTA. The TCA/PTA mixture also precipitates proteins readily in the presence of 0.1% Tween 20, Triton X-100, or Nonidet P-40. TCA/PTA has the capacity to precipitate a wider cross-section of proteins than TCA alone, the combination of acids remaining effective in the presence of SDS and the other detergents examined in this study.

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