avoid self-igation is not required, and transformants could be easily isolated without using X-Gal and IPTG. In addition, pTOC-KR had excellent cloning efficiency in various types of E. coli strains, such as E. coli XL1-blue, BL21, DH5α, JM109, and JM110, and showed its potential broad host-range character in other types of E. coli.

ACKNOWLEDGMENTS

We wish to express our appreciation to Dr. D.R. Helinski, who kindly provided pAS12 and pRR46. This work was supported by Kyung Hee University, Korea.

REFERENCES


Cost-benefit analysis of a method using diatomaceous earth to purify Tamm-Horsfall protein

Jaideep Shenoi1, Biji T. Kurien2, Sadamu Kurono3, Ranjan Mascarenhas3, Hiroyuki Matsumoto3, and Robert H. Scofield2,3

1University of Oklahoma Health Sciences Center, Tulsa, 2Oklahoma Medical Research Foundation, Oklahoma City, and 3University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA


Ever since Tamm and Horsfall first identified Tamm-Horsfall protein (THP) over a half-century before, its true function has baffled investigators (1). Studies have suggested that THP could have a role in prevention of urinary tract infection (UTI) by its adherence properties (2,3). THP is synthesized in the thick ascending kidney tubules and is not found anywhere else in the body, although the THP gene is evolutionarily conserved in all vertebrates (4). THP exists as a polymeric glycoprotein with a monomeric molecular weight (MW) of 88,000, and storage of THP has always proven to be difficult. THP quantitatively decreases over time even when dissolved in phosphate buffer at 4°C. This phenomenon was prevented when using Triton®X-100 and EDTA at an alkaline pH (TEA) instead of phosphate buffer (5).

The cost of commercial THP is $155/100 µg (Biomedical Technologies, Stoughton, MA, USA). Currently, THP can be purified using two different published methods. The original method described by Tamm and Horsfall (1950) is time-consuming (1). The second method, by Serafini-Cessi et al. (6), uses diatomaceous earth and then utilizes deionized water for the desorption of THP. This results in a reduced time of 4–6 h to purify the same amount of THP from urine, excluding dialysis and lyophilization.

Our aim was to utilize inexpensive and freely available materials to develop an uncomplicated and reproducible procedure with a shorter time frame. Molecular weight markers were obtained from Invitrogen (Carlsbad, CA, USA). The sodium dodecyl sulfate (SDS)-polyacrylamide precast gel (12%) was obtained from ISC BioExpress (Kaysville, UT, USA), and the filtering agent Celite® 521 and the diatomaceous earth (DE) were both from Sigma (St. Louis, MO, USA). DE was also obtained from Best Prices Storable Foods (Dallas, TX, USA). All other reagents were of analytical grade.

THP was purified using the most recent and quick method available (6).

Large volumes of 1.5 L of overnight urine were collected and neutralized with 10 M NaOH. It was then filtered through 20 g of DE layered on a Whatman no. 1
filter (Whatman, Clifton, NJ, USA). The filter was washed with 1 L phosphate-buffered saline (PBS). The DE on the filter was scraped off, and the earth was suspended in 150 mL of water for 30 min and then centrifuged at 20,000×g for 20 min. The pellet was discarded. To the supernatant, 0.14 M NaCl and 0.025 M phosphate buffer, pH 7.5, was added. The solution was filtered through DE again, and the filter was washed with 100 mL PBS. The earth was scraped off from the filter and suspended in 50 mL of water for 30 min and then centrifuged at 20,000×g for 20 min. The pellet was discarded. The supernatant was dialyzed against water and lyophilized to obtain THP. THP was then weighed from the same amount of urine (1.5 L). THP could be dissolved in phosphate buffer, pH 7.4, or TEA solution mixed at 4°C for 15 min and then centrifuged at 14,000×g for 15 min before the supernatant is used as a pure reconstituted form of THP.

Two modifications to this method were explored: (i) the urine was not neutralized with 10 M NaOH; and (ii) DE was mixed with the urine for a 15-min period before filtering the DE/urine mixture through Whatman filter paper no.1, which was placed on a Buchner funnel with vacuum suction.

SDS-polyacrylamide gel (12%) elec-

<table>
<thead>
<tr>
<th>Experiment (No.)</th>
<th>DE Used</th>
<th>Method</th>
<th>Amount THP/1.5 L Urine (mg)</th>
<th>Cost/25 g of DE Used ($)</th>
<th>Cost/g of THP Purified ($)</th>
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<tr>
<td>1</td>
<td>DE b</td>
<td>Unmodified</td>
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<td>22.50</td>
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<td>DE b</td>
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<td>DE c</td>
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<td>0.08</td>
<td>0.001</td>
</tr>
</tbody>
</table>

aSerafini-Cessi et al. (6).
bFrom Sigma.
cFrom Best Priced Storable Foods.

THP, Tamm-Horsfall protein; DE, diatomaceous earth.

Cost was calculated by multiplying the yield and the cost for the different forms of DE used for each experiment and extrapolating the results to identify the cost per gram of THP purified. As no other reagents were used, DE was the only tangible factor taken into account. Note: (i) the cost of commercial THP is $155/100 µg (approximately $1,550,000/g); (ii) experiment 2a was done by the modified Serafini-Cessi method (6), but the urine was neutralized; while (iii) in experiment 2b, the urine was not neutralized.
trophoresis and silver nitrate staining were carried out by standard methods and were used to identify the purity of THP. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) of THP was used to further confirm the purity of THP (7).

We prepared pure THP by the published method of Serafini-Cessi et al. (6) and also by our modified method. We compared the yield and cost per gram of THP recovered between the two methods and when using different forms of DE in the modified method (Table 1).

Single bands corresponding to 88 kDa on a SDS-polyacrylamide gel (12%) electrophoresis and silver nitrate staining identified the purity of THP in the different experiments (Figure 1). Even though similar concentrations of purified THP (1 mg/mL) were loaded in each of the wells, the 88-kDa bands were of different intensity. This pattern continued to emerge repeatedly. Similar peaks between commercial THP and Celite 521-purified THP confirmed purity by mass spectrometric analysis.

The Tamm and Horsfall method uses NaCl to precipitate THP and takes 1 week to purify THP from 2 to 3 L of urine, excluding dialysis and lyophilization. This prompted us to use the method by Serrafini-Cessi et al. (6), which utilized deionized water for the desorption of THP to occur from DE, with a reduced time of 4–6 h. The drawbacks of method by Serrafini-Cessi et al. (6) are several. Time is needed for the DE to settle over the filter paper without vacuum suction, so as not to break the layer, and additional time is consumed scraping the DE off the filter paper after urine has been passed through the filter. The layer often breaks apart while pouring the urine and also while scraping the DE off the filter paper later. The time needed to layer DE and pass urine through it is decreased by one-third when DE is mixed in urine before layering it over the filter paper while suction is being used. The time needed to scrape the DE from the filter paper is decreased by 50% as the DE layer peels off as a complete pack without crumbling apart. There was no significant change in the amount of THP obtained with non-neutralization of urine. In summary, using DE, large volumes of overnight urine (1.5–3 L) can be used to obtain a 32% increased quantity of THP in half the time (2 to 3 h) of currently available procedures, excluding dialysis and lyophilization. By using Celite 521, a less expensive purified form of DE and a larger increase of 133% of purified THP were obtained.

When we used a nonpurified form of DE (obtained from Best Priced Storable Foods), we obtained a 29.13% increase in purified THP. SDS-polyacrylamide gel electrophoresis and silver nitrate staining of gel showed that the THP was pure. The difference in intensity of protein bands in Figure 1 is not an artifact of gel loading, since equal concentrations (1 mg/mL) of protein were loaded. We discovered that THP undergoes autolysis (unpublished observation), and we hypothesize that Celite 521 inhibits this activity and that this accounts for the elevated protein levels seen during purification of THP using Celite 521. Similar peaks in the mass spectrometric analysis of THP further confirmed the purity of THP. Because no additives are added, the filtered urine may be used later for the purification of other urinary proteins, enzymes, and hormones.

We purified THP by the published method of Serafini-Cessi et al. (6) and also by our modified method, and we then compared both the yield and the cost involved between these methods and also to the commercial THP ($155/100 µg). Not only was our modified method an easier procedure, it was also cost-effective and provided a higher yield when compared to purified THP by the Serafini-Cessi method and to commercial THP. The cost per gram of THP recovered was $2184.47 by the Serafini-Cessi method compared to our modified method with DE (from Sigma) to purify THP, in which the cost per gram of THP recovered was $1654.41/g. The cost per gram of pure THP recovered from purification further decreased to $83.30 when Celite 521 was used instead of DE. Using a nonlaboratory-grade unpurified form of DE, although the yield was less, the cost per gram of THP recovered was a tenth of a cent.

Our modified method, therefore, is a rapid, inexpensive, and reproducible method to obtain pure laboratory-grade THP. We also propose that instead of lyophilizing the dialysate and then needing to redisolve the THP in buffer, the dialysate itself may be used as a pure preparation of THP for laboratory use.

REFERENCES


