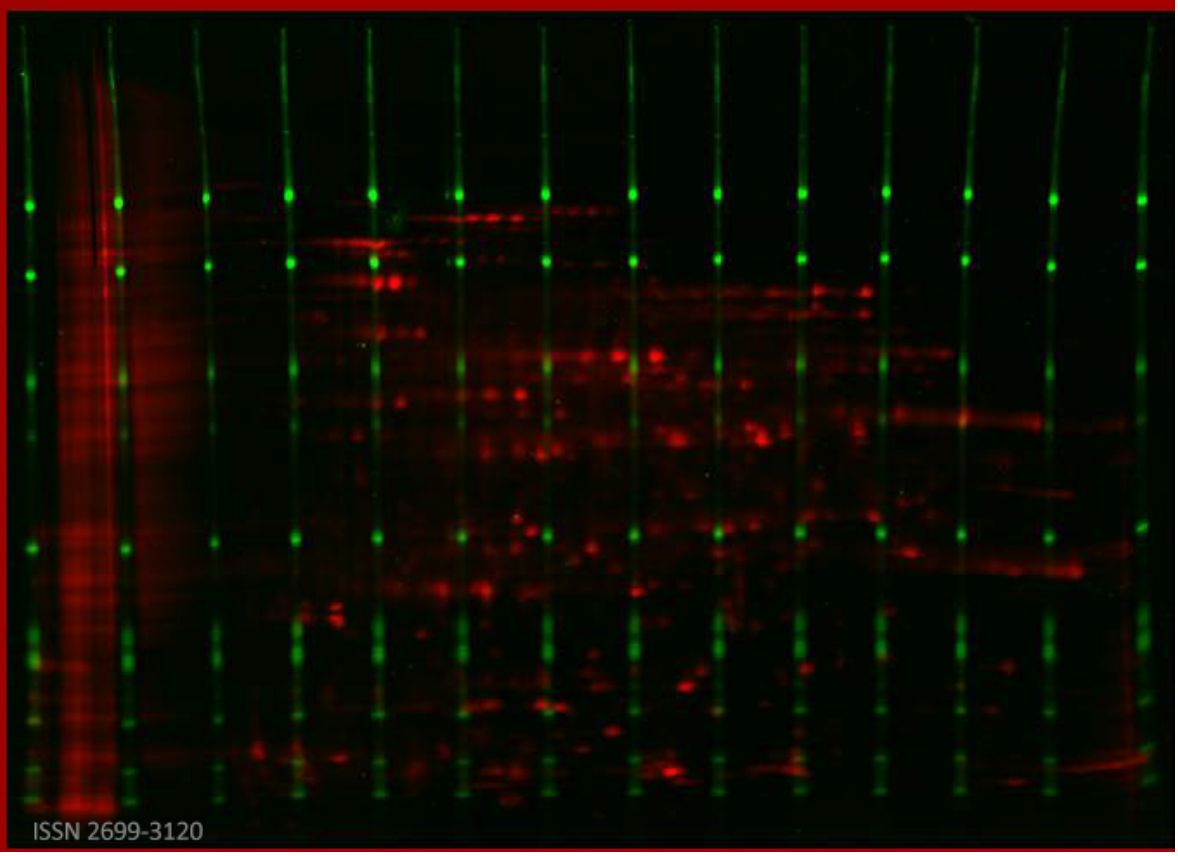
Volume 5 · Number 1 · August 2021

MERCATOR Journal Biomolecular Analysis

Laboratory Protocols

Mass spectrometry & Proteomics

Editor Simone König



MERCATOR Journal Biomolecular Analysis Vol 5 · No 1 · August 2021 · pp 1-3

ISSN 2699-3120

Digital laboratory notes of the Core Unit Proteomics CUP
Interdisciplinary Center for Clinical Research IZKF
Medical Faculty
Westphalian Wilhelms University of Münster
Germany
https://campus.uni-muenster.de/cu-proteomics
Edited by Simone König
CUP/IZKF, Röntgenstr. 21, 48149 Münster
koenigs@uni-muenster.de
Ph. +49-251-8357164

Cover image
Mercator gel (run by D. Ackermann at CUP) representing the award-winning CoFGE technology for standardized gel electrophoresis

Protocol

Albumin aggregation and the re-solubilization of dried serum proteins

Shahid Aziz[1,2], Rabaab Zahra[1], Anna Maria Berg[3], Doreen Ackermann[3], Faisal Rasheed[1,2], Simone König[3#]

[1] Quaid-i-Azam University, Islamabad, Pakistan

[2] Pakistan Institute of Nuclear Science and Technology, Islamabad, Pakistan

[3] Core Unit Proteomics, Interdisciplinary Center for Clinical Research, University of Münster

[#]Corresponding author: koenigs@uni-muenster.de

Abstract

Albumin is known for its aggregation. As it is a major component of serum, it may be largely responsible for the difficulties in re-suspending dried serum proteins. We found it necessary to break up the solid pellet using a ball mill and subsequently a chaotropic agent and an organic solvent to generate a homogenous protein solution suitable for mass spectrometry-based protein expression analysis.

Introduction

Bovine serum albumin (BSA) is often used as a reference compound in protein concentration determination. We have noted that at BSA concentrations of ~50 mg/ml and above in buffer containing 8 M urea, 0.1% (w/v) sodium dodecylsulfate (SDS), 10 mM tris (2-carboxyethyl) phosphine hydrochloride (TCEP), 100 mM TrisBase, a stiff gel forms having a consistency unlike other protein solutions of this concentration. The reason is likely a particular interaction of BSA with the buffer components, namely the detergents, possibly forming liquid crystals, because BSA is known to aggregate and form mixed micelles with surfactants [1, 2]. Urea was also found to

support aggregation [3]. Prior studies have always been performed in water or simple phosphate buffers so that the physico-chemistry of this particular aggregation remains to be properly explored. Nevertheless, the BSA-detergent interaction has been already successfully used in liquid-crystal-based biosensors [4].
The fact that serum makes up more than half of the total protein content in plasma [5, 6] may be responsible for the observation that it was a difficult task to re-dissolve serum proteins, which have been prepared in lysis buffer (8 M urea, 100 mM TrisBase, 2% (w/v) SDS, 10 mM TCEP) and dried for cross-border transport. Simply adding water to reconstitute the original protein solution resulted in an inhomogenous gelatinous lump even when the volume was drastically increased. As it was very important to generate a homogenous protein solution for downstream processing, we have tried different means of solubilization and found one which can be recommended for subsequent experiments such as protein identification and expression analysis not requiring maintenance of protein activity.

Experimental

Blood samples were centrifuged and the serum was collected. Serum (250 and 500 µl) was processed with 500 µl or 1 ml, respectively, lysis buffer (8 M urea, 100 mM TrisBase, 2% w/v SDS) and centrifuged at 16.000 x g for 5 min. The supernatant was pipetted into a new sample tube and 100 µl TCEP (10 mM in H_2O) were added. After vortexing and centrifugation (5 min, 16.000 x g) the supernatant was lyophilized in a new tube using a speedvac concentrator and stored at -80 °C until further use. To re-solubilize the dried serum proteins, samples were treated with a volume of Milli-Q water equivalent to the original volume of the supernatant. However, a firm gelatinous consistency formed, which was stable, even when the water amount was doubled. Prolonged shaking, slight heating to 33 °C, ultrasonic treatment, and the addition of different solubilizing substances such as small alcohols and acetonitrile (ACN), did not help. Only the protocol given below generated a homogeneous protein solution.

Protocol
The dry pellet was treated by means of Vibrating Mixer Mill Type MM 300 (Retsch) for 20 min at 30 Hz (one 5 mm stainless steel grinding ball, no buffer added) to break up the solid structure. Milli-Q water (1 ml) containing 20 % acetonitrile and 1 M guanidine hydrochloride [7] was added and the sample was vortexed (2.000 min^{-1}, 1 h, Vibrax). This procedure generated a milky homogenous emulsion which could be further processed by filter-aided sample preparation for mass spectrometric analysis with no loss in protein identification sensitivity.

Conclusion

A method to re-solubilize dried serum proteins was developed for later filter-based digestion and protein expression analysis. Simple re-solubilization in water was impossible as a result of protein aggregation. Following destruction of the dry protein pellet using a ball mill, the addition of both ACN and the chaotropic agent guanidine hydrochloride was necessary to generate a homogenous emulsion which could be successfully processed further. Certainly, this procedure is of no use to biochemical experiments which require proper protein folding and preserved activity. Therefore, in general, serum should be used as is without too many intermittent handling steps.

References

[1] Misra PK, Dash U, Maharana S. Investigation of bovine serum albumin-surfactant aggregation and its physicochemical characteristics, Coll Surf A: Physicochemical and Engineering Aspects. 2015; 483: 36-44.
[2] Amin S, Barnett GV, Pathak JA, Roberts CJ, Sarangapani PS. Protein aggregation, particle formation, characterization & rheology. Curr Opin Coll Interf Sci. 2014; 19: 438-449.
[3] Roughton BC, Iyer LK, Bertelsen E, Topp EM, Camardad KV. Protein aggregation and lyophilization: Protein structural descriptors as predictors of aggregation propensity. Comput Chem Eng. 2013; 58: 369–377.
[4] Amin NU, Siddiqi HM, Kun Lin Y, Hussain Z, Majeed N. Bovine serum albumin protein-based liquid crystal biosensors for optical detection of toxic heavy metals in water. Sensors (Basel). 2020; 20: 298.
[5] Busher JT. Serum Albumin and Globulin. In: Walker HK, Hall WD, Hurst JW, editors. Clinical Methods: The History, Physical, and Laboratory Examinations. 3rd edition. Boston: Butterworths; 1990. Chapter 101.
[6] Moman RN, Gupta N, Varacallo M. Physiology, Albumin. [Updated 2021 Aug 9]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021
[7] Smith JS, Scholtz JM. Guanidine hydrochloride unfolding of peptide helices: Separation of denaturant and salt effects. Biochemistry 1996; 35: 7292-7297.