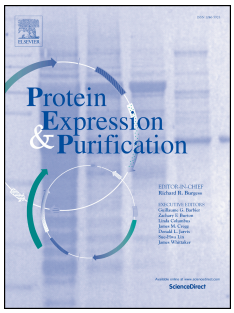
Accepted Manuscript



Efficient protease based purification of recombinant matrix metalloprotease-1 in *E. coli*

Lokender Kumar, Warren Colomb, John Czerski, Christopher R. Cox, Susanta K. Sarkar

PII: S1046-5928(18)30087-1

DOI: 10.1016/j.pep.2018.04.001

Reference: YPREP 5247

To appear in: *Protein Expression and Purification*

Received Date: 9 February 2018

Revised Date: 24 March 2018

Accepted Date: 3 April 2018

Please cite this article as: L. Kumar, W. Colomb, J. Czerski, C.R. Cox, S.K. Sarkar, Efficient protease based purification of recombinant matrix metalloprotease-1 in *E. coli*, *Protein Expression and Purification* (2018), doi: 10.1016/j.pep.2018.04.001.

Efficient protease based purification of recombinant matrix metalloprotease-1 in *E. coli*

Lokender Kumar[1], Warren Colomb[1], John Czerski[1], Christopher R. Cox[2], and Susanta K. Sarkar [1,*]

[1]*Single Molecule Biophysics Laboratory, Department of Physics, Colorado School of Mines, 1500 Illinois Street, Golden, CO 80401, USA*

[2]*Advanced Biodetection Technology Laboratory, Department of Chemistry, Colorado School of Mines, 1500 Illinois Street, Golden, CO 80401, USA*

Corresponding author:

[*]ssarkar@mines.edu

Abstract

MMP1 is an essential enzyme for tissue remodeling both in normal and pathological states. We report a method of purifying activated human MMP1 in *E. coli* without using urea or 4-Aminophenylmercuric acetate (APMA). Instead, a non-ionic detergent, Triton X-100, was used in the lysis buffer to solubilize MMP1 followed by the protease activities of both trypsin and MMP1 to digest *E. coli* proteins and activate pro-MMP1. Identity of activated MMP1 was confirmed by Western blot using anti-human MMP1 antibodies, whereas the mass was determined to be 43 kD using matrix assisted laser desorption ionization time-of-flight mass spectrometry (MALDI TOF-MS). Collagen and gelatin degradation by purified MMP1 were confirmed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE) of degraded FITC-labeled type-1 collagen and gelatin zymogram. Broad-spectrum protease activity of purified MMP1 was also confirmed by lysis of native *E. coli* proteins. Inexpensive high throughput purification of recombinant human MMP1 in *E. coli* will enable easier MMP1 production for diverse applications.

Keywords: Recombinant human MMP1, Protease activity of MMP1, Collagen degradation, Gelatin zymography.