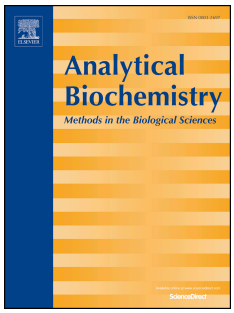
Accepted Manuscript



Synthesis and purification of linkage-specific polyubiquitin chains of distinct length for structural studies

Anshumali Mittal, Binita Shakya

PII: S0003-2697(18)30834-0

DOI: 10.1016/j.ab.2018.08.007

Reference: YABIO 13102

To appear in: *Analytical Biochemistry*

Received Date: 22 January 2018

Accepted Date: 8 August 2018

Please cite this article as: A. Mittal, B. Shakya, Synthesis and purification of linkage-specific polyubiquitin chains of distinct length for structural studies, *Analytical Biochemistry* (2018), doi: 10.1016/j.ab.2018.08.007.

Synthesis and purification of linkage-specific polyubiquitin chains of distinct length for structural studies

Anshumali Mittal*[1,2], Binita Shakya[1]

[1]Department of Biochemistry, University of Utah School of Medicine, Salt Lake City, USA

[2]Present Address- Department of Molecular Biology and Microbiology, Tufts University School of Medicine, Boston, USA

*Correspondence: Anshumali.Mittal@tufts.edu

Abstract

Polyubiquitylation is one of the most versatile post-translational modifications involved in the regulation of numerous intracellular signaling processes. An assembly procedure that is simple, robust, and efficient to synthesize and purify linkage-specific polyubiquitin chains of defined length at a preparative scale is required in biophysical and structural studies. Here, we have optimized known enzymatic procedures in the form of a protocol to obtain multi-milligrams of Lys48- and Lys63-linked polyubiquitin chain types with more than 99% purity. Mass spectrometry (ESI/MS) analysis of K48- and K63-linked diubiquitin confirmed that the enzymes used in the preparation generated homogeneous linkages with no promiscuity.

Introduction

Covalent conjugation of one or more ubiquitin moieties to a protein is referred to as ubiquitylation [1], with attachment of a single ubiquitin called monoubiquitylation and attachment of a chain (or tree) of ubiquitin moieties called polyubiquitylation. In a polyubiquitin chain, ubiquitin molecules are connected to each other by covalent bonds between the carboxyl group of the C terminus of one ubiquitin and the ε-amino group of one of the seven Lys residues (Lys6, Lys11, Lys27, Lys29, Lys33, Lys48 and Lys63) in the neighboring ubiquitin or through the ubiquitin amino terminal Met1 residues generating the linear chain. Quantitative mass spectrometric analysis of ubiquitin conjugates of *S. cerevisiae* lysate have revealed that all possible linkage types coexist in cells [2]. Ubiquitin polymers of different linkages adopt different topologies and are associated with distinct biological functions, deciding between life and death of proteins. For example, extensively studied K48- and K11-linked polyubiquitin