QUALITATIVE AND QUANTITATIVE ANALYSES OF β-TUBULIN Cva (C-TERMINAL VARIABLE ACIDIC AMINO ACID REGION) BY MALDI-TOF MASS-SPECTROMETRY

SHOJI OKAMURA*, KAZUHIRO KUDO, AND TSUNEO IMANAKA
Department of Biological Chemistry, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, Toyama 930-0194, Japan

Abstract. The C-terminal region of tubulin is highly acidic and its amino acid sequence is divergent among the isotypes. This region is also known to have various types of post-translational modifications. Comprehensive analyses of C-terminal proteolytic fragments of tubulin by matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF MS) will be useful in understanding the role of this region in microtubule function during cell proliferation and differentiation. Lysylendopeptidase (LysC)- and endoproteinase AspN (AspN)-digested fragments were effective for predicting/identifying the isoforms in a complex mixture. It was also shown that tobacco BY2 cells were labeled efficiently by 15N-containing medium. The stable isotope-labeled components were detected as separate peaks from their 14N-counterparts with their masses increased as predicted by their nitrogen contents, and this method is useful for the quantitation of specific fragments.

Keywords: β-Tubulin isoform, C-terminal region, MALDI-TOF MS, stable isotope, tobacco BY2

Abbreviations: AspN = endoproteinase AspN, Cva = C-terminal variable acidic amino acid region, DAC = diammonium citrate, LysC = lysylendopeptidase, HNM = heavy Nagata’s medium, IPG = immobilized pH gradient, MALDI-TOF MS = matrix-assisted laser desorption/ionization-time of flight mass spectrometry, NM = Nagata’s

*Author to whom correspondence should be addressed: Department of Biological Chemistry, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, Toyama 930-0194, Japan; phone: +81-76-434-7547; e-mail: okamura@pha.u-toyama.ac.jp
medium, PTM = post-translational modification, THAP = trihydroxyacetophenone, TTLL = tubulin tyrosine ligase-like

1. Introduction

1.1. BACKGROUND

Plant cell culture has been considered to be an excellent system for investigating the control mechanism of cell division and differentiation. Plant tissue de-differentiates into callus when it is grown on an appropriate medium containing auxin. The callus is a kind of stem cell and can differentiate into a whole plant. Recently, receptors for plant hormones were found and the basic mechanism of tissue development seems to be quite similar to the patterning during early development of animal embryos. Plant callus cells generally grow in a completely defined medium. This is a great advantage over animal cell culture, which usually grows in a medium containing uncharacterized serum, when studying cell cycle events and differentiation.

Patterning at the root differentiation accompanies with the cessation of proliferation and initiation of cell elongation. This process proceeds with close relation to the auxin gradient. The translocation of signal molecules into nuclei is thought to be mediated by microtubules, but the mechanism for the process is not fully elucidated. Carrot GD2 cells were shown to change its mitotic activity and cell shape after the medium auxin was changed from 2,4-D to IAA. This phenomenon was thought to be a model for the switching from proliferation to elongation mode at the early patterning of root tissue. In both of the process of cell division and elongation, microtubule distribution and its function change dramatically. The main microtubule protein subunits, \( \alpha \)-, \( \beta \)-tubulin, consist of multiple isoforms in multicellular organisms. Tubulin was isolated from carrot and its properties were studied. The composition of isoforms seems to be different according to the physiological state of the cells or differentiated tissues. Carrot GD2 cells were quite useful for the biochemical studies. However, they are not suited for cell cycle study because they can not be synchronized well by aphidicolin. However, tobacco BY2 cells can be fairly well synchronized for cell cycle events. In this chapter, we describe the experiments performed mainly on BY2 cells.