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Metal-binding proteins scanning and determination by combining gel electrophoresis, synchrotron radiation X-ray fluorescence and atomic spectrometry

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Abstract

In the present work, protein bands from in vitro embriogenic *callus* (*Citrus sinensis* L. Osbeck) were investigated using micro-synchrotron radiation X-ray fluorescence (μSR-XRF) after sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) separation. Metal-binding protein quantification was done after microwave oven decomposition of gel by synchrotron radiation total reflection X-ray fluorescence (SR-TXRF), flame atomic absorption spectrometry (FAAS) and flame atomic emission spectrometry (FAES). According to the analysis of the protein bands, it is possible to observe that both 81 and ca. 14 kDa proteins present different Fe signal intensity at different positions. The analysis of 53 kDa protein, showed even more interesting results. Besides Fe, the μSR-XRF experiments indicate the presence of Ca, Cu, K and Zn. Chemical elements such as Cu, K, Fe and Zn were determined by SR-TXRF, Mg by FAAS and Na by FAES. Ca was determined by SR-TXRF and FAAS only for accuracy check. In the mineralised protein bands of 81 and around 14 kDa band, only Fe was determined (105 and 21.8 μg g^{-1}). For those protein bands (86—ca. 14 kDa)

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were determined, Ca, K, Cu and Zn in a wide concentration range (42.4–283, 2.47–96.8, 0.91–15.9 and 3.39–29.7 μg g^{-1}, respectively).

Keywords: Metal-binding proteins; Metal determination; SDS-PAGE; Synchrotron radiation; FAAS and FAES

1. Introduction

The determination of signalling-metals related to cell differentiation and plant growth is of great interest for biochemical studies [1,2].

Among metals, calcium is one of the most important in the regulation of plant growth and development, being known as an important signalling-agent in biochemical and physiological processes [3]. These events take place at the cellular level, such as cell division and enlargement, and others [4,5]. In addition, due to its high affinity with calmodulin and other proteins, calcium can act directly in many cellular processes, modulating the function and activity of other proteins [3], and mediating processes such as polarized growth, mitosis, cytoplasm streaming, among others [6].

Only few studies have related the mineral composition of plant material to its effect in biochemical events [7–9]. Due to the importance of these metals in biochemical processes, their quantification is of utmost importance for clarifying interactions between metals and proteins in those events already commented.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) is the technique most widely used for protein separation in biological samples. This technique in association with particle-induced X-ray emission (PIXE) was applied for in situ Fe localization in iron–sulphur protein (HiPiP) analysis [10], as well as Fe and Ni identification and determination in the hydrogenase enzyme from a bacterium [11].

Metal-binding proteins quantification was only recently reported. Chéry et al. [12] presented a method for proteins separation by SDS-PAGE, followed by Se determination in each protein band using electrothermal vaporization inductively coupled plasma mass spectrometry (ETV-ICP-MS). The reported detection limit was 50 pg of selenium per piece of gel.

Chen et al. [13] obtained a Se speciation in subcellular fractions of human liver proteins. SDS-PAGE was used for protein separation followed by Se determination employing hydride generation-atomic fluorescence spectrometry (HG-AFS).

An in situ detection of metalloproteins in human liver cytosol method by synchrotron radiation X-ray fluorescence (SR-XRF), was reported by Gao et al. [14,15]. The authors pointed out for some advantages of this method, such as the absence of sample pre-treatment and some operational characteristics: non-destructive and high sensitivity. In this research, however, this method was not applied for metal-binding protein determination.

In the present work, protein from in vitro plant material samples of *Citrus sinensis* L. Osbeck were separated by SDS-PAGE and the metal-binding proteins were scanned using micro synchrotron radiation X-ray fluorescence (μSR-XRF). Comparing with other works