PARTICLE-INDUCED X-RAY EMISSION (PIXE): A TOOL FOR MULTIELEMENT ANALYSIS IN THE YEAST CELLS[a]

Ileana Cornelia Farcasanu*, F. Nishiyama and T. Miyakawa*****

abstract: The analytical particle-induced X-ray emission (PIXE) procedure for the multielement analysis offers great potential for trace element analysis in the biological and medical fields. Here we present the PIXE procedure as an accurate tool to detect both the major and the trace chemical elements within the *Saccharomyces cerevisiae* cells.

Introduction

One of the main characteristics of living organisms is their ability to maintain certain parameters within the physiological limits. Out of the basic homeostasis mechanisms, regulation of the ratio between chemical elements must be an important determinant for adaptation to various environments. Many elements, both major and trace elements [1, 2], take part in the complicated biochemical reactions and one cannot say beforehand which one has a crucial role in certain case. It is also known that there exist synergetic effects, such that the concentration of certain element influences the function of another. If one has a complete elemental profile, there is less chance of overlooking valuable information.

Particle-induced X-ray emission (PIXE) analysis is a multielement technique that has been developing worldwide over the past years [3-6]. Certain advantages make it useful for applications in biological sciences: 1) PIXE offers its maximum sensitivity or minimum detection limit in two atomic number regions, $20 < Z < 35$ and $75 < Z < 85$; the former region happens to contain the majority of trace elements of interest in various areas of biological sciences. 2) PIXE analysis also determines several major elements such as K, Ca, S and P, and it is often of great interest to know their concentrations. 3) Optimum sensitivities are attained at rather low proton energies; this is important since it means that small accelerators are most suitable for PIXE, with consequent benefits in both reliability and economics. 4) Analysis times are typically a few minutes in duration. 5) The

---

[a] This work was performed at Hiroshima University, Japan
* Bucharest University, Faculty of Chemistry, Department of Organic Chemistry and Biochemistry, Sos. Panduri 90-92, Bucharest, Romania. Tel.: 40-21-4103178/ext.135. E-mail: ifarcasanu@chem.unibuc.ro. Corresponding author
** Department of Power Engineering and Applied Physics, Graduate School of Engineering, Hiroshima University, Japan
*** Department of Molecular Biotechnology, Graduate School of Advanced Sciences of Matter, Hiroshima University, Japan

Analele Universităţii din Bucureşti – Chimie, Anul XIII (serie nouă), vol. I-II, pag. 39-43

multielement nature of PIXE in combination with high sensitivity permits the determination of many elements in a single run.

Trace metals (Fe, Zn, Cu, Mn, Co) play an important role in cell metabolism, especially as coenzymes and cofactors in various cellular processes. Usually, the environmental concentrations of such elements are low and cells must develop active transport processes to accumulate them. On the other hand, when the internal concentration of these metals gets higher than the physiological levels, they become toxic, mainly by non-specific binding to proteins or by interference to other metals' metabolism. Under such circumstances, cells develop defense strategies, by limiting the influx, by sequestering the excess cations in a non-toxic form, or by activating the export. PIXE was used to monitory the uptake or efflux rates of individual trace elements [7, 8] as well as the intracellular distribution of manganese [9], in the *Saccharomyces cerevisiae* cells, but no multielement analysis was carried out.

In this article we present PIXE as a way to measure highly encountered elements (K, S, P, Mg) along with trace elements (Fe, Mn, Zn, Ca, Cu, Ni, Co) inside the cells of the yeast *Saccharomyces cerevisiae* grown under standard conditions.

Experimental

Strains, media, and preparation of the PIXE samples

The brewer's yeast *Saccharomyces cerevisiae*, strain W303 1A (*MAT* **a** *trp1 leu2 ade2 ura3 his3 can1-100*) was used for our experiments. Cells were grown in liquid YPD (yeast extract-polypeptone-dextrose) [10] supplemented with adenine (400 μg/ml) and uracil (200 μg/ml). A preculture was used to inoculate the culture at a concentration of about 2×10^5 cells/ml. The cell suspensions were incubated with shaking for 8 hours at 28ºC. Cells were harvested by centrifugation and were washed three times with deionized water. Finally, cells were brought to the desired density by adding an appropriate volume of deionized water. All centrifugation (3 min, 3000 x g) was done at 4ºC.

PIXE experiments

Suspensions with various cell densities were placed in aliquots of approximately 5 μl on polycarbonate membranes (Nucleopore Corp., U. S. A., pore size 0.1 μm, thickness ~ 6 μm) and let dry overnight in a desiccator containing silicagel. The elements in the cellular material were determined using a Van de Graaff accelerator (Nissin High Voltage Co. Ltd., model AN-2500). The proton energy used was 2 MeV for heavy elements ($Z > 18$), but for detection of light elements ($Z < 18$), the proton energy was degraded to 0.75 MeV in order to obtain a good signal-to-noise ratio. For Mg, P and S determinations, a Si (Li) X-ray detector (Canberra SL80175) with 25 μm beryllium window was used. For K, Ca, Mn, Fe, Ni, Cu, Zn determinations, a Ge (int) X-ray detector (ORTEC GLP-10180/07) with 127 μm beryllium window was used. All data were accumulated by a multichannel pulse height analyzer AMS-1000 (Laboratory Equipment Corp.). The elements determined were expressed as μg/mg total cellular protein; total cellular protein content was determined by

the method described by Bradford [11]. The measurements were carried out in five different experiments, using triplicates for every sample.

Results and discussion

A typical example of a PIXE spectrum of the main elements present in the yeast cell are presented in Fig. 1. The amounts of various elements determined by PIXE inside the *S. cerevisiae* cells are presented in Table 1. Minimal detection limit within an organic matrix is considered to be 10^{-7}~10^{-6} mM [3]. If the yeast cell is considered to be a sphere of 5μ diameter, then for detecting for example Mn^{2+}, whose average concentration in the cell is around 5 mM [12], the minimal cell density in the sample should be of the 10^6 order. We found for the majority of elements detected that the cell density 10^5-10^6 cells/sample gave accurate and reproducible values (Table 1). However, when the cell density was higher than 5 x 10^6, the reproducibility was poor, probably because of the interference of the cell matrix.

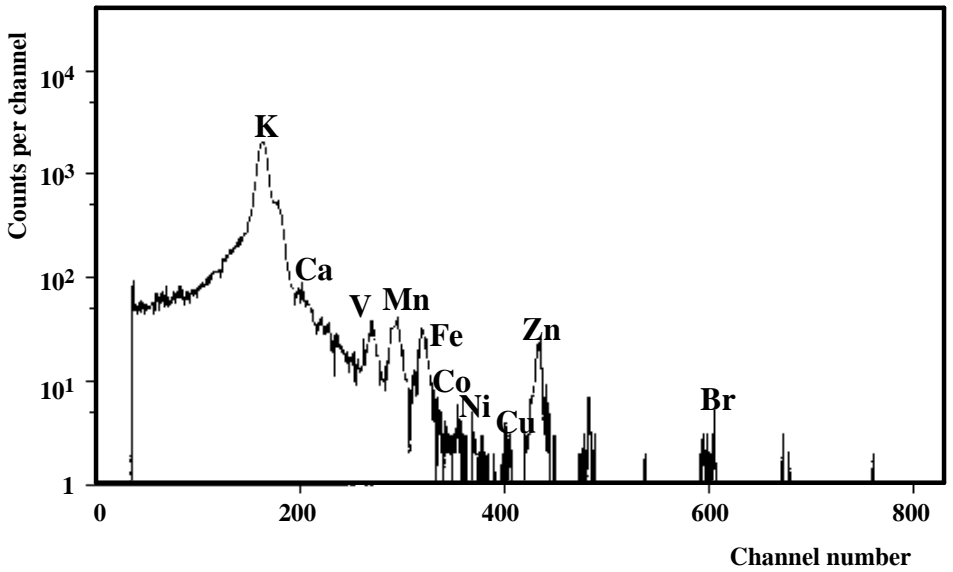

Fig. 1. ***A PIXE spectrum of elements found in yeast cell.***
The yeast cells were grown to the log phase and concentrated by centrifugation. 5 μl of cell suspension (10^6 cells, in this case) were used for PIXE analysis as described in the **Experimental** section. One typical PIXE spectrum is presented.

Certain trace elements (Fe, Cu, Zn) were easy to determine by PIXE (Fig. 1), but in order to measure some of the minor trace elements (e. g., Co, Ni,) it was necessary to optimize the sensitivity. This was done by superposing two different spectral data.

The lightest element that could be detected in our experiments was Na, but the cellular content determined increased with sample dilution and reproducibility of results was poor. This observation suggested us that for light elements (Li, Na) other analytical techniques (e. g., atomic spectroscopy) may be more appropriate.

Table 1. The element composition of yeast cells as determined by PIXE. The results are the average of five different measurements.

Element	Cellular content (μg/mg protein)	OCD[1] (cells/sample)[2]
Mg	21.93±0.57	$1x10^5$~$1x10^6$
P	167.5±5.95	$1x10^5$~$1x10^6$
S	19.56±0.93	$2x10^5$~$1x10^6$
K	169.77±15.23	$1x10^5$~$1x10^6$
Ca	2.33±0.15	$2x10^5$~$1x10^6$
Mn[3]	0.025±0.015	$1x10^6$~$5x10^6$
Fe	0.355±0.005	$2x10^5$~$5x10^6$
Co[4]	$1.4x10^{-2}$	$5x10^6$
Ni[4]	$2.7x10^{-3}$	$5x10^6$
Cu	0.122±0.003	$1x10^6$~$5x10^6$
Zn	2.26±0.02	$2x10^5$~$5x10^6$
Br[4]	$1.3x10^{-2}$	$5x10^6$
Rb[3]	0.033±0.001	$1x10^6$~$5x10^6$

[1]OCD = optimal cell density; [2]Sample volume = 5μl;[3] Minimal cell density for detection: 1x 10^6 cells/ml; [4] Minimal cell density for detection: $5x10^6$ cells/ml.

REFERENCES

1. Hughes, M. N. (1990) The Inorganic Chemistry of Biological Processes, Chichester: John Wiley
2. Frausto da Silva, J. J. R. and Williams, R. J. P. (1993) **The Biological Chemistry of the Elements**, Oxford: Oxford University Press.
3. Johansson, S. A. E. and Campbell, J. L. (1988) **PIXE: a novel technique for elemental analysis**, John Wiley and Sons Ltd.
4. Nygren, O. (2002) New approaches for assessment of occupational exposure to metals using on-site measurements, *J Environ Monit*. **4**: 623-7.
5. Hou, X, Peters, HL, Yang, Z, Wagner, KA, Batchelor, JD, Daniel, MM, and Jones, BT. (2003) Determination of trace metals in drinking water using solid-phase extraction disks and X-ray fluorescence spectrometry, *Appl Spectrosc*. **57**: 338-42.
6. Ebara, M, Fukuda, H, Hatano, R, Yoshikawa, M, Sugiura, N, Saisho, H, Kondo, F. and Yukawa, M. (2003) Metal contents in the liver of patients with chronic liver disease caused by hepatitis C virus. Reference to hepatocellular carcinoma, *Oncology* **65**, 323-30.
7. Farcasanu, IC, Hirata, D, Tsuchiya, E, Nishiyama, F. and Miyakawa, T. (1995) Protein phosphatase 2B of Saccharomyces cerevisiae is required for tolerance to manganese, in blocking the entry of ions into the cells, *Eur J Biochem*. **232**, 712-7.
8. Farcasanu, IC, Mizunuma, M, Hirata, D. and Miyakawa T. (1998) Involvement of histidine permease (Hip1p) of Saccharomyces cerevisiae in Mn^{2+} transport, *Mol. Gen. Gen*. **259**, 541-8.
9. Farcasanu IC, Hirata D, Tsuchiya E, Mizuta K and Miyakawa T. (1999). Involvement of thioredoxin peroxidase type II (Ahp1p) of Saccharomyces cerevisiae in Mn^{2+} homeostasis, *Biosci Biotechnol Biochem*. **63**, 1871-81.

10. Sherman, F., Fink, G. R. and Hicks, J. B. (1986) **Methods in yeast genetics**, Cold Sprig Harbor Laboratory Press, Cold Spring Harbor, New York

11. Bradford, M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding, *Anal. Biochem*. **72**, 248-54.

12. Okorokov, L. A., Lichko, L. P., Kadomtseva, V. M., Kholodenko, V. P., Titovski, V. T. and Kulaev, I. S. (1977) Energy-dependent transport of manganese into yeast cells and distribution of accumulated ions. Eur. *J. Biochem*. **75**, 373-7.