Determination of trace elements in hepatic cells of Zn-deficient mice by instrumental neutron activation and PIXE analyses (II)

Junji Kamishima, Ryuji Minayoshi, Michiko Kawashima[*1], Hideo Suganuma[*1], Motoko Noguchi[*2], Shoji Futatsugawa[*3], Kouichiro Sera[*4] and Makoto Yanaga[*1]

Graduate School of Science and Engineering, Shizuoka University
836 Ohya, Suruga-ku, Shizuoka 422-8529, Japan

[*1] Radiochemistry Research Laboratory, Faculty of Science, Shizuoka University
836 Ohya, Suruga-ku, Shizuoka 422-8529, Japan

[*2] Department of Biology and Geosciences, Faculty of Science, Shizuoka University
836 Ohya, Suruga-ku, Shizuoka 422-8529, Japan

[*3] Radioisotope section, Japan Radioisotope Association
2-28-45 Honkomagome, Bunkyo, Tokyo 113-8941, Japan

[*4] Cyclotron Research Center, Iwate Medical University
348-58 Tomegamori, Takizawa 020-0173, Japan

Abstract

The concentrations of trace elements in hepatic subcellulars and cytosolic protein of zinc deficient mice were determined in order to investigate the behavior and role of zinc and other trace elements.

Eight-week-old male mice of ICR strain were divided into two groups; one was fed with zinc deficient diet (<1 μg/g Zn), the other with control diet (30 μg/g Zn). After 3 weeks of treatment periods, their livers were removed. Two types of experiments were performed. In the first experiment, the liver samples homogenized with HEPES buffer which adjusted to pH 7.4 with $KHCO_3$ were centrifuged under differential conditions in order to separate into cellular fragments and 5 subcellular fractions, such as nuclear, mitochondrial, lysosomal, microsomal and cytosolic fractions. Each fraction was freeze-dried for instrumental neutron activation analysis (INAA). Concentrations of 11 elements, Na, Mg, Cl, Mn, Fe, Co, Cu, Zn, Se, Br, and Rb, were determined by INAA. In the second experiment, sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and silver stain was performed for cytosolic fraction of other mice. After electrophoresis, the gel was cut into protein bands and subjected to PIXE analysis.

Almost all of the trace elements except for iron investigated in the present study mainly existed in cytosol which contains various proteins and enzymes. The zinc concentration in three fractions of zinc deficient mice was lower than that of control ones. Especially, in the cytosolic fraction, the difference of zinc concentration between both groups was remarkable. On the other hand, cobalt concentrations in all hepatic

subcellular fractions of zinc deficient mice increased significantly compared with control mice. These results suggested that metal proteins and other compounds, in which zinc was replaced by cobalt, might partially be synthesized in the liver of zinc deficient mice. It was also suggested that the other metal elements might slightly substitute for zinc in zinc binding proteins. Furthermore, concentrations of trace elements in each protein band were determined by PIXE analysis and zinc concentration in each band standardized with the silver concentration, i. e., normalized with the protein amount. From this result, there are no significant differences in almost all bands between zinc deficient mice and control ones. Therefore, it is considered that zinc binding proteins were decreased (or disappeared) under zinc deficient condition.