

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

Makoto Yanaga, Takanori Ogi^{*1}, Nobuyuki Kinugawa^{*1}, Ryuji Minayoshi^{*1}, Junji Kamishima
Motoko Noguchi^{*2}, Hideo Suganuma, Shoji Futatsugawa^{*3}, Kouichiro Sera^{*4}

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

^{*1}Department of Biology and Geosciences, Faculty of Science, Shizuoka University
836 Ohya, Suruga-ku, Shizuoka 422-8529, Japan

^{*2}Graduate School of Science and Engineering, Shizuoka University
836 Ohya, Suruga-ku, Shizuoka 422-8529, Japan

^{*3}Nishina Memorial Cyclotron Center, Japan Radioisotope Association
348-58 Tomegamori, Takizawa 020-0173, Japan

^{*4}Cyclotron Research Center, Iwate Medical University
348-58 Tomegamori, Takizawa 020-0173, Japan

Abstract

The effect of zinc deficiency on concentrations of trace elements in hepatic cells of mice was investigated by means of INAA combined with cell fractionation technique.

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 by KHCO₃ 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, Mg, Mn, Na, Cl, Br, Cu, Se, Rb, Fe, Zn and Co, were determined by INAA. In the second experiment, sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed for cytosolic fraction of another mice.

Almost all of the trace elements except for iron investigated in the present study mainly existed in cytosol which contains various proteins and enzymes. Zinc concentrations in each cell organelle (each hepatocellular fraction separated by centrifugation) of zinc deficient mice examined were not distinctly lower

than those of control mice except for cytosolic fraction. On the other hand, cobalt concentrations in all organelles 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. However, when the positions and number of the protein bands on gel developed the cytosolic fraction of zinc deficient mice were compared with those of control ones, no significant differences were found between them.