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Zinc as an Essential Trace Element in the Acceleration of Matrix Vecilles-Mediated Mineral Deposition

Atsushi KAWAKUBO, Tsunenori MATSUNAGA, Hidetaka ISHIZAKI, Yoshihiko HAYASHI*
Department of Cariology, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki 852-8588, Japan

Introduction

The effect of Zn on the function of matrix vesicles (MVs) remains controversial. The present study was designed to investigate the effect of Zn on the alkaline phosphatase (ALP) activity of osteoblasts and in the initial biological MVs-mediated mineral deposition. For the direct analyses of the low level of Ca present in MVs preparation, synchrotron radiation analyses could be used as a nondestructive method with a detection limit of below the 10 ppm range. Furthermore, the effect of Zn on the transformation of calcium phosphate was analyzed using a SEM fitted with EDX.

Materials and Methods

Osteoblasts (NOS-I cells) were treated with varying concentrations ($1x10^{-6}$ to $1x10^{-3}$ *M*) of Zn dissolved in culture medium. After three, five, and seven days of culture, ALP activity was assayed. NOS-1 cells were seeded at a density of $1x10^{6}$ cells. After seven days of culture without Zn, they were retrieved, and MVs were isolated using a collagenase digestion and ultracentrifugation method. For the detected of a low level of calcium concentration in MVs, X-ray fluorescence (XRF) analyses were applied (BL-4A). The effect of Zn for transformation of calcium phosphate was analyzed using a scanning electron microscope fitted with an energy dispersive X-ray microanalysis (EDX) system.

Results and Discussion

ALP is attached to the external surface of plasma membranes by phosphoethanolamine bound to oligosaccharides., where it hydrolyzes phosphate esters to increase the phosphate concentration and mineralization of the external matrix. ALP is also a Zn metalloenzyme and contains two molecules of Zn. The removal of the Zn results in the loss of ALP activity.

The ALP activity of osteoblasts in culture medium supplemented with $1x10^{-5}$ *M* of Zn was significantly increased at both five and seven days compared with other concentrations ($P<0.01$).

There was a statistically significant difference between both variations factors: the supplementation with or without Zn, and the examination date ($P<0.001$). XRF data demonstrated higher levels of calcium concentration over time in the Zn-supplemented group.

There was a statistically significant difference between both variation factors: the supplementation with or without Zn, and examination date ($P<0.01$). EDX data showed that mineral deposits beginning on day 3 were transformed from whitlockite to calcium phosphate near hydroxyapatite, and that Zn accelerated this transcormation.

XRF analysis of Ca

Ca concentrations (mean ± SD; ppm)		
Zn	-	+
1 day	9.089 ± 1.164	10.925 ± 0.789
3 days	17.365 ± 0.285	21.385 ± 1.516
5 days	25.883 ± 2.249	40.737 ± 7.483

Data are from triplicate samples.

Zn may play an important role in osteoblast mineralization through intra- and extracellular Zn movements involving a Zn storage protein, and Zn transporters might mediate the effects of Zn on osteoblast mineralization

The proper concentration of Zn increased the ALP activity of osteoblasts after five and seven days of incubation. The present XRF and EDX data suggest that the increase of mineral deposition with Zn exposure for one to five days might be mediated by the activation of ALP and calcium-binding proteins.

References

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* hayashi@nagasaki-u.ac.jp