Flow-injection analysis of zinc(II) ions with an ALP-column based on an apoenzyme reactivation method and application of the method to regulation of the column activity.

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A regulation method of an enzyme activity by its cofactor of the enzyme was developed, which was based on an apoenzyme reactivation method [1-3]. Applying this method to alkaline phosphatase (ALP), the activity of ALP was controlled proportionally with concentrations of zinc (II) ions ranging from 0 to about 80 % using the same enzyme column per se.

Alkaline phosphatase (orthophosphoric monoester phosphohydrolase, EC 3.1.3.1, from *Escherichia coli*, 70 U/mg powder), a kind of typical metalloenzymes, of which cofactor was zinc (II) ion, was generously provided by Asahi Chemical Industry Co., Ltd., and was covalently immobilized onto inner wall of gold capillary (1.5 mm (outer diameter) x 1.0 mm (inner diameter) x 10 cm (length)) modified with 2-aminoethanethiol and then, introduced into an FIA system. The FIA system used in this study was illustrated in Fig. 1. This FIA system was assembled with the plunger pump, rotary injection valves, the enzyme column, and a flow-through quartz cell attached to a UV/VIS detector. A 100 mM 2,6-pyridine dicarboxylate solutions (pH 7.0) containing 10 mM citrate and 1.0 M NaCl as cofactor-complexing agents for removing metal ions from the enzymes and various concentration of *p*-nitrophenyl phosphate (PNPP, 0.1 ml) as the substrate were injected via rotary injection valves, separately. Tris-HCl buffers (0.1 M, pH 8.0, 1.0 M NaCl, 0.1 μM citrate) were continuously pumped through the system.

A 100 μl of PNPP solution with various concentrations was injected into the FIA system, and an changes in absorbance at 405 nm attributable to *p*-nitrophenol generated in hydrolysis by ALP was monitored. Increase in absorbance was observed by injecting the PNPP solution into the immobilized enzyme column. When the substrate solution was injected to the once chelating agent exposed-column, absorbance at 405 nm in the effluents from the enzyme column decreased significantly. Then, adding trace amount of standard zinc(II) ions to the system reactivated the column. The enzyme activity of the column was sufficiently recovered by injecting 1.0 mM standard zinc(II) ions. Reversible changes in the enzyme activity due to injections of the chelators and also standard metals could be observed repeatedly. Responses attributable to the partly reactivated apoenzymes differed from concentrations of zinc(II) ions injected to the enzyme column. A correlation between the concentration of zinc(II) ions (0 – 5.0 μM) and reactivation ratio (0 – 80 %) of the ALP column was obtained. Thus, the activity of ALP could be regulated by injecting various concentrations of the cofactor.

[1] I. Satoh, M. Sekine, Y. Iida, *Proc. 37th Chemical Sensor Sym.*, 19, Supple. B, p.127-129 (2003).
[2] I. Satoh, M. Sekine, Y. Iida, *Proc. 36th Chemical Sensor Sym.*, 19, Supple. A, p.37-39 (2003).
[3] Y. Iida, C. Gonokami, T. Morii, I. Satoh, *Electrochemistry*, **71**, 453-456 (2003).

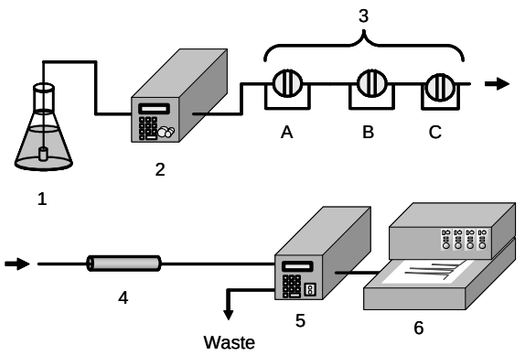

Fig. 1 Schematic diagram of the FIA system.
1) Carrier reservoir, 2) Pump, 3) Rotary injection valves with the sample loop [A) for chelator, B) for standard zinc (II) ions, C) for substrate for assay of an activity of the ALP column], 4) The enzyme column, 5) UV/VIS detector (λ: 405 nm), 6) recorder.

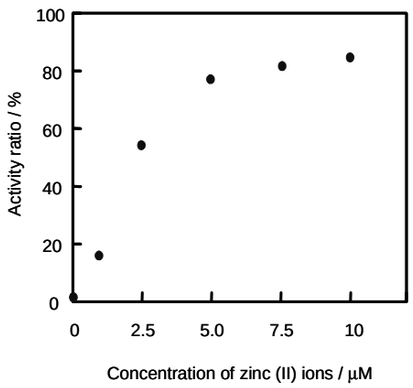

Fig. 2 Relationship between concentration of zinc (II) ions and activity of ALP column.