Specific Recognition and Detection of Phosphorylated Proteins using Characteristic of Metal Ion

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Phosphorylation is a fundamental covalent post-translational modification that regulates the function, localization, and binding specificity of target proteins. Organisms utilize this reversible reaction of proteins to control many cellular activities, including signal transduction, apoptosis, gene expression, cell cycle progression, cytoskeletal regulation, and energy metabolism. Abnormal protein phosphorylations are deeply related to carcinogenesis and neuropathogenesis. Methods for monitoring the phosphorylation status of proteins are thus very important with respect to the evaluation of diverse biological and pathological processes.

Recently, we reported that a dinuclear metal complex of 1,3-bis[bis(pyridin-2-ylmethyl)-amino]propan-2-olato acts as a novel phosphate-binding tag molecule, Phos-tag, in an aqueous solution under physiological conditions[1)]. The Phos-tag has a vacancy on two metal ions that is suitable for the access of a phosphomonoester dianion (R-OPO_3^{2-}) as a bridging ligand. The resulting 1:1 phosphate-binding complex, R-OPO_3^{2-}–(Phos-tag)$^{3+}$, has a total charge of +1. A dinuclear zinc(II) complex (Zn^{2+}–Phos-tag) strongly binds to phenyl phosphate dianion (K_d = 2.5 × 10^{-8} M) at a neutral pH. The anion selectivity indexes against SO_4^{2-}, CH_3COO^-, Cl^-, and the bisphenyl phosphate monoanion at 25 ˚C are 5.2 × 10^3, 1.6 × 10^4, 8.0 × 10^5, and >2 × 10^6, respectively. A manganese(II) homologue (Mn^{2+}–Phos-tag) can also capture R-OPO_3^{2-} anion, such as phosphoserine, phosphotyrosine, or phosphohistidine, at alkaline pH of *ca*. 9. By utilizing the Phos-tag molecule and its derivatives, we here introduce convenient and reliable methods for the detection of phosphorylated proteins, such as phosphate-affinity chromatography[2, 3)] or phosphate-affinity electrophoresis[3)]. We believe that our Phos-tag technology developed using characteristic of metal ion would result in great progress in phosphoproteomics.

[References]

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