Development of New Ratiometric Zinc Fluorescent Probes

Masayasu Taki[1,2], Yukio Yamamoto[1]

[1]Graduate School of Human and Environmental Studies, Kyoto University, [2]Department of Natural Resources, Graduate School of Global Environmental Studies, Kyoto University

New Zn(II)-specific fluorescent probes, ZnSAID-1 and ZnSAID-2, have been synthesized and characterized in order to clarify the roles of chelatable Zn^{2+} in biological systems. Both ZnSAID-1 and ZnSAID-2 employ a phenylindol modified with a sulfonamide group as a fluorophore, that would be expected red-shift in the emission spectrum when the sulfonamide is depronated by addition of metal ion. ZnSAIDs were obtained in a multistep synthesis in which the phenylindol core was prepared through the ring-closing reaction of stilbene derivative by heating in triethyl phosphite at 125 ˚C for 4 h. The absorption spectrum of ZnSAID-2 showed a characteristic band at 342 nm under physiological conditions (50 mM HEPES, pH 7.20, 0.1 M KNO_3). Upon addition of an equivalent of Zn^{2+}, the absorption peak shifted to 352 nm without an isosbestic point. When excited at 342 nm, the metal-free form exhibits an emission band at 427 nm that shifts to 466 nm by complexation with Zn^{2+}. Such a large shift in the absorption and emission spectra suggests that the deprotonated sulfonamide anion coordinates to Zn^{2+} in neutral solution. The metal binding stereochemistry of ZnSAID-1 was evaluated by the X-ray crystallographic analysis of $[Zn(L)]PF_6 \bullet 2H_2O$, where L is L = (2-*p*-toluenesulfonylamino) phenoxy-2-(di(2-picolyl)aminomethyl)phenoxyethane. $[Zn(L)]PF_6 \bullet 2H_2O$ was prepeared by treating the ligand with an equimolar amount of $Zn(ClO_4)_2 \bullet 6H_2O$ and Et_3N in methanol, and the following an anion exchange with $NaPF_6$. The crystal structure reveals a 1:1 stoichiometry with Zn^{2+} wherein one oxygen atom and four nitrogen atoms from DPA moiety and sulfonamide anion bound to the Zn^{2+} as expected.

Detailed characterizations of ZnSAID-1 and ZnSAID-2 including fluorescent quantum yield, Zn^{2+} specificity, and the dissociation constant for Zn^{2+} will be discussed.