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A Novel Potassium Sensing in Aqueous Media with a Synthetic Oligonucleotide Derivative. Fluorescence Resonance Energy Transfer Associated with Guanine Quartet−Potassium Ion Complex Formation

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Potassium ion (K^+) plays an important role in biological systems together with sodium, calcium, and other metal ions, and therefore the development of a method to visualize K^+ in a cell is very important. For one thing, K^+ is involved in the maintenance of extracellular osmolarity in conjugation with sodium ion (Na^+), and the concentration of K^+ in the living cell is associated with regulation of concentrations of other ions such as Ca^{2+} and Cl^- which are transported across the plasma membrane.[1] Recently, it is perceived that a deficiency of the K^+ channel binding protein is responsible for the onset of an irregular heartbeat.[2] Although numerous studies were reported concerning K^+ sensors, virtually all of them were based on a heterogeneous system such as an ion carrier ingrained membrane.[3] Detection of K^+ in a homogeneous system is limited to potassium-binding benzofuran isophthalate (PBFI)[4] and coumarin diacid cryptand [2.2.2] (CD222)[5] as a water-soluble fluoroionophore, but K^+ selectivity against Na^+ was low in both systems. Yamauchi et al. developed a system coupled with B15C5 crown ether and γ-cyclodextrin (γ-CD) to detect K^+ with high discrimination ability for K^+ against Na^+.[6] However, B15C5 as fluoroionophore is only sparingly soluble in water, and an excess of γ-CD and a small amount of organic solvent were still necessary, and is not applicable to the K^+ detection or determination in living cells.

Herein a novel potassium sensing oligonucleotide (PSO, Figure 1) was constructed which can detect K^+ in water. The oligonucleotide contains four GGG sequence sites that offer a unique K^+ binding site in the intramolecular tetraplex folding to form a guanine quartet. The guanine quartet is in fact a part of the tetraplex structure of DNA carrying the sequence observed at the termini of the eukaryotic chromosome (telomere DNA sequence).[7] Tetraplex formation is known to be promoted by the presence of monovalent cations.[8] Especially K^+ is able to stablize the guanine quartet.[9] This happens by a coincidence of the size of K^+ with the cavity created between two guanine quartets. Because the distance of both termini of the oligonucleotide carrying telomere sequence changes dramatically upon formation of a tetraplex structure from a random coil and its formation is strongly dependent on the concentration of K^+, potassium sensing may be performed when two chromophores which can undergo fluorescence resonance energy transfer (FRET) are attached to the termini of the telomere oligonucleotide.

PSO (Sigma Genosys) carries a part of the human telomere sequence, d(GGGTTAGGGTTAGGGTTAGGG), with two fluorophores, 6-carboxyfluorescein (6-FAM) and 6-carboxytetramethylrhodamine (6-TAMRA), at the 5′- and 3′-termini of the oligonucleotide as the donor and acceptor, respectively. Conditions were set where this oligonucleotide can form a tetraplex structure in the presence of K^+ and the two chromophores come close to each other to bring about FRET, as shown in Figure 1, and therefore K^+ can be detected from the magnitude of FRET.

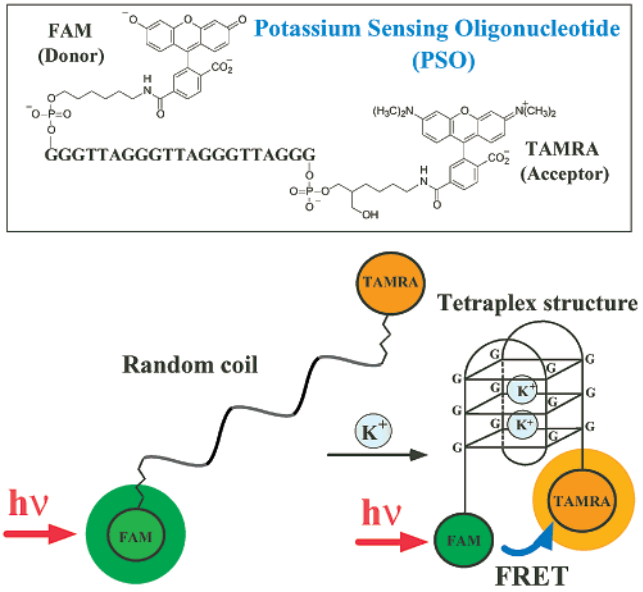

Figure 1. Chemical structure of PSO and its schematic tetraplex structure induced by K^+ resulting in a FRET change.

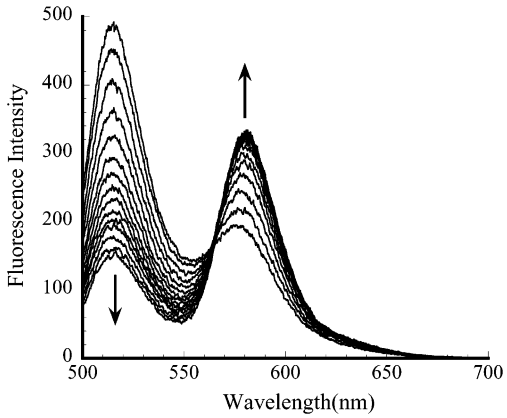

Figure 2. Fluorescence change of 0.21 μM PSO upon addition of a various amount of K^+ in 5 mM Tris-HCl (pH 7.0) at 25 °C. λ_{ex} = 492 nm.

Figure 2 shows a fluorescence change of PSO upon addition of K^+. The fluorescence intensity at 515 nm corresponding to 6-FAM decreased, whereas that at 581 nm of 6-TAMRA increased upon addition of K^+, and a clear isoemissive point appeared at 565 nm. This phenomenon indicates that PSO has only two states of extended (random coil) and folded forms (tetraplex) in the aqueous

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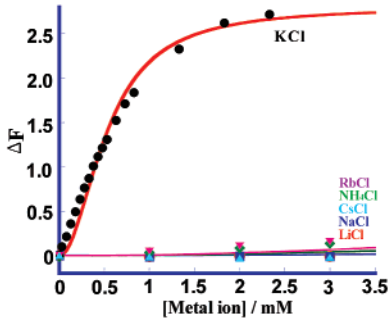

Figure 3. ΔF ($F - F_0$) of 0.21 μM PSO upon addition of a various amount of selected cations in 5 mM Tris-HCl (pH 7.0) at 25 °C.

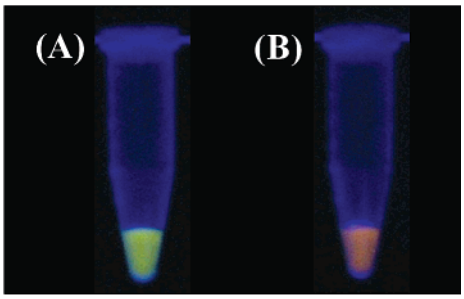

Figure 4. Fluorescence image of 2 μM PSO solution in the absence (A) and presence of 15 mM KCl (B) in 5 mM Tris-HCl (pH 9.0) at 25 °C. PSO was excited at 302 nm with a UV transilluminator.

solution and that the presence of K^+ shifts the equilibrium toward the tetraplex form. ΔF ($=F - F_0$) was plotted against the concentration of K^+, Rb^+, NH_4^+, Cs^+, Na^+, and Li^+, as shown in Figure 3. Here, F ($=F_{581}/F_{515}$) is the ratio of the fluorescence intensities at 581 and 515 nm and represents the magnitude of FRET, and F_0 is the value of F in the absence of K^+. PSO responded only to K^+ in the concentration range from 0 to 2.5 mM, although it responded to NH_4^+, Na^+, and Rb^+ as well at higher concentrations of 300 mM. PSO did not respond to Li^+ and Cs^+.

When two cations (M^+) are assumed to bind to one PSO like the model in Figure 1, the following equation is formulated:

$$\Delta F = (\Delta F_\infty [M^+]_o^2 K_{ass})/(1 + [M^+]_o^2 K_{ass}) \quad (1)$$

$$K_{ass} = [PSO - M_2]/[PSO][M^+]^2 \quad (2)$$

where K_{ass} is the binding constant, $[M^+]_o$ is the initial concentration of M^+, and $\Delta F_\infty = F_\infty - F_o$ (the subscripts ∞ and o define the bound (tetraplex) and free (extended) forms of PSO, respectively). The K_{ass} was obtained by fitting eq 1 to the plot of ΔF against various concentrations of monovalent cations. In all of the cases, good fits were obtained by assuming that two cations bind with PSO. This is in agreement with the model where K^+ is incorporated into the cavity created between two guanine quartets. The K_{ass}'s of PSO for monovalent cations thus obtained were as follows: $(1.3 \pm 0.2) \times 10^7$, $(3.0 \pm 0) \times 10^2$, $(1.5 \pm 0.1) \times 10^3$, and $(3.4 \pm 0.2) \times 10^3$ M^{-2} for KCl, NaCl, NH_4Cl, and RbCl, respectively. The K_{ass}'s for LiCl and CsCl were not obtained because no FRET was observed with them. Because the order of K_{ass} parallels that of the stability of the G-quartet structure in the presence of a cation, the fluorescence change observed should reflect the formation of a G-quartet with the cation. The selectivity of PSO for K^+ against Na^+ is 43 000 times, the highest ever reported, to the best of the authors' knowledge.

Detection of K^+ at submicromolar levels was possible under optimal conditions. Circular dichroism (CD) spectra of PSO in the absence and presence of K^+ showed that these fluoroscence changes were due to a structural change from a random coil to a tetraplex of PSO, as revealed by the identity of the CD spectra in the presence of K^+ with that of the tetraplex structure.[10]

K^+ in the test tube was visualized with PSO under the irradiation with a UV transilluminator ($\lambda_{ex} = 302$ nm). The yellow fluorescence of PSO turned to red in 5 mM Tris-HCl (pH 9.0) upon addition of 15 mM KCl as shown in Figure 4, whereas no fluorescence change was observable at pH 7.0 because both of the fluorophores are excited. Divalent cations such as Mg^{2+} or Ca^{2+} are also known to influence the quartet structure of the telomere oligonucleotide,[11] and in fact only a fluorescence decrease was observed in their presence. This should derive from the formation of a parallel tetraplex to result in fluorescence quenching. It thus seems certain that the K^+ concentration can be monitored with PSO even near the physiological condition (5 mM Tris-HCl (pH7.0) containing 145 mM NaCl, 1.5 mM $MgCl_2$, and 2.5 mM $CaCl_2$ at 25 °C).

These results suggested that PSO might be applicable to the K^+ detection in living cells. The present novel finding will help improve remarkably the detection of K^+ in the presence of excess Na^+ in homogeneous aqueous medium near physiological conditions, although the effect of divalent ions should be taken into account when applying to in vivo assay. The concept of the molecular design is also hoped to help develop a field of supramolecular sensing material taking advantage of nucleotide interaction.[12]

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Supporting Information Available: TOF-mass data and CD spectra of PSO, curve-fitting data for the fluorescence change upon addition of a various amount of K^+ or $Na,^+$ and fluorescence change of PSO near the physiological condition (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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