262 nm (3800). CCDC-190226 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or deposit@ccdc.cam.ac.uk).

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Naked-Eye Detection of Phosphate Ions in Water at Physiological pH: A Remarkably Selective and Easy-To-Assemble Colorimetric Phosphate-Sensing Probe**

Min Su Han and Dong H. Kim*

Phosphate anions are one of most important constituents of living systems. Together with heterocyclic bases and sugars, phosphates make up the genes, the hereditary elements of living systems. In addition, phosphate ions and their derivatives play pivotal roles in signal transduction and energy storage in biological systems.[1] Numerous sensors for anions, including phosphate ions, have been devised, but most of them use organic solvents as the detection medium because these sensors rely on hydrogen-bonding and electrostatic interactions for the recognition of analytes.[2] These interactions are, however, attenuated drastically in a highly polar medium such as water, because of the competing solvation effect.[3] The detection of anions such as phosphate in water is, hence, a challenging task.[4] We report herein a colorimetric sensor that can detect phosphate anions in an aqueous solution of neutral pH values. The sensor is easy to assemble and shows a high sensitivity and excellent selectivity for phosphate ions over other anions.

In assembling the sensor, we took advantage of metal–ligand interactions. Such interactions are so highly favorable that they occur even in polar media. Furthermore, the metal ion can present some geometrical preferences, thus imparting selective binding tendencies towards anions of a given shape.[5] 2,6-Bis(bis(2-pyridylmethyl)aminomethyl)-4-methylphenol (H-bpmp) was reported to form a crystalline dinuclear complex with Co^{II},[6] and Seo et al. reported that the phenylphosphonate anion binds to the dinuclear complex of Co^{III} with H-bpmp by bridging the two metal ions.[7] These reports led us to explore the metal complex of H-bpmp as a receptor for phosphate ions. The dinuclear Zn^{II} complex of H-bpmp was readily obtained by dissolving H-bpmp and zinc perchlorate in water; the complex is colorless and has a good water solubility, which are features desirable for using the complex as a receptor for a water-soluble chemosensor. We chose pyrocatechol violet, a catechol-type pH-sensitive dye, as the chromogenic indicator for the sensor. Catechols are known to coordinate to the two metal ions in a phenoxo-bridged binuclear metal complex.[8] Furthermore, it is known that the yellow color of pyrocatechol violet at neutral pH may change to blue when it binds to a metal ion.[9] Therefore, the displacement of the receptor-bound pyrocatechol violet by a phosphate anion would be communicated visually as well as spectrophotometrically. The competition approach[10] used for assembling the present sensor is schematically illustrated in Figure 1.

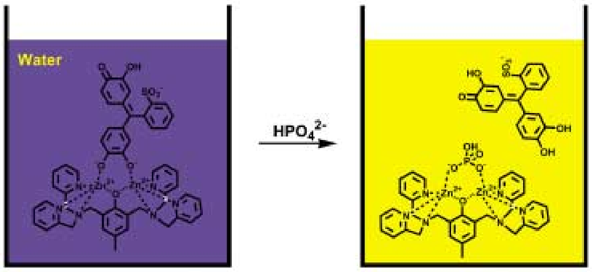

Figure 1. A schematic representation of the phosphate anion sensor.

The sensing ensemble was prepared by simply mixing H-bpmp,[11] zinc perchlorate, and pyrocatechol violet[12] in a 1:2:1 molar ratio in an aqueous solution of 10 mM HEPES buffer pH 7.0 (HEPES = 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid). Figure 2a shows the UV/Vis spectra obtained when the solution of $[Zn_2(H\text{-bpmp})]^{3+}$ was titrated into the aqueous buffer (pH 7.0) solution of the indicator (50 µM). The color change from yellow ($\lambda_{max} = 444$ nm) to blue ($\lambda_{max} = 624$ nm) observed upon the addition of $[Zn_2(H\text{-bpmp})]^{3+}$ is ascribed to the binding of pyrocatechol violet to the Zn^{II} ions

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[*] Prof. D. H. Kim, M. S. Han
Center for Integrated Molecular Systems
Division of Molecular and Life Sciences
Pohang University of Science and Technology
San 31 Hyojadong, Pohang 790-784 (Korea)
Fax: (+82) 54-279-5877 or (+82) 54-279-8142
E-mail: dhkim@postech.ac.kr

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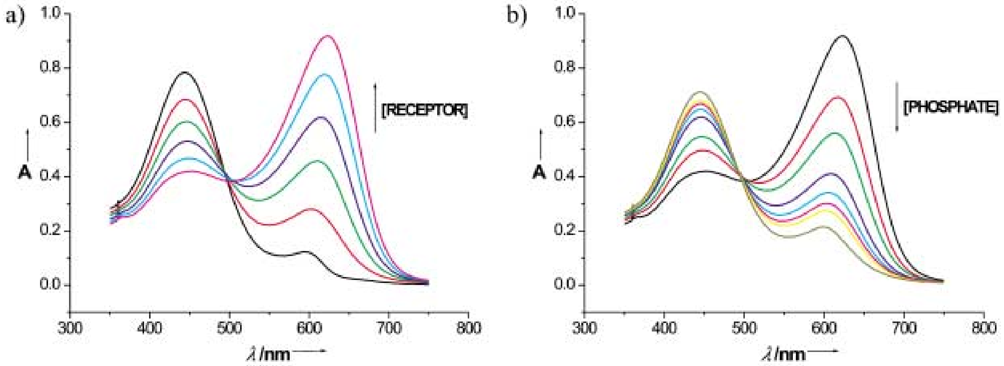

Figure 2. a) UV/Vis spectra obtained by additions of $[Zn_2(H\text{-}bpmp)]^{3+}$ solution (final concentrations: 0, 10, 20, 30, 40, and 50 μM) to the pH 7.0 aqueous buffer (HEPES, 10 mM) containing pyrocatechol violet (50 μM); b) UV/Vis spectra obtained by additions of HPO_4^{2-} solution (final concentrations: 0, 25, 50, 100, 150, 200, 250, and 500 μM) to the pH 7.0 aqueous buffer (HEPES, 10 mM) containing $[Zn_2(H\text{-}bpmp)(\text{pyrocatechol violet})]^{+}$ (50 μM).

in the receptor complex to form $[Zn_2(H\text{-}bpmp)(\text{pyrocatechol violet})]^{+}$. As expected, the addition of phosphate anions to the aqueous solution of the ensemble resulted in a change of the color from blue to yellow, and caused changes in the UV/Vis absorption spectra: the peak at $\lambda_{max} = 624$ nm decreases while the peak at 444 nm increases (Figure 2b).[13] The effect of the pH value of the medium on the phosphate ion sensing was studied, and we found that the UV/Vis absorbance changes described above occur in the pH range of 6.5–7.5, but most distinctively at pH 7.0.[14] The thermodynamic parameters and kinetic constants for the bindings of the indicator as well as the analyte to the $[Zn_2(H\text{-}bpmp)]^{3+}$ were determined[14] by isothermal titration calorimetry (ITC).[15] It can be seen from Table 1 that the phosphate anion binds the receptor over twofold more tightly than the indicator and the binding is enthalpically driven, which suggests that there are strong coordinative interactions. The large unfavorable entropic contribution to the binding of the indicator compared to that of the phosphate anion suggests that ordering of water molecules at the complex interface in the binding may occur.

Table 1. Thermodynamic parameters and association constants (K_{ass}) for the binding of pyrocatechol violet or HPO_4^{2-} ions to $[Zn_2(H\text{-}bpmp)]^{3+}$ in an aqueous solution of pH 7.0 (HEPES).

Data obtained from ITC experiment	Pyrocatechol violet	HPO_4^{2-}
ΔH° (kcal mol^{-1})	-11.83 ± 0.12	-7.55 ± 0.08
ΔG° (kcal mol^{-1})	-6.56	-7.01
ΔS° (e.u.)	-17.39	-1.79
K_{ass} (M^{-1})	$(5.3 \pm 0.2) \times 10^4$	$(11.2 \pm 0.8) \times 10^4$

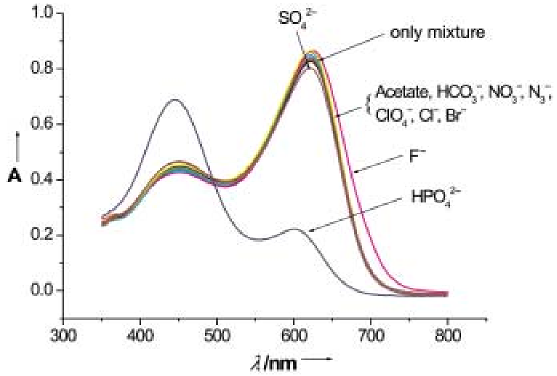

Figure 3. UV/Vis spectra of the $[Zn_2(H\text{-}bpmp)]^{3+}$– pyrocatechol violet mixture (50 μM in a pH 7.0 aqueous solution) in the presence of various anions (250 μM).

Figure 4. The color of the mixture (50 μM) in the absence and presence of anions (250 μM): from left to right; no anion, $CH_3CO_2^-$, HPO_4^{2-}, HCO_3^-, NO_3^-, N_3^-, ClO_4^-, SO_4^{2-}, F^-, Cl^-, and Br^-.

The present sensor exhibits excellent selectivity towards phosphate ions over other anions including sulfate and fluoride ions (Figure 3). This is remarkable because virtually no sensor reported to date can detect phosphate ions with such a high selectivity in an aqueous solution.[16] Sensors that can detect analytes by the naked eye, without resorting to any spectrometer, is of particular interest because of its convenience. Only a few such sensors have, however, been reported.[17] The use of the present ensemble for such a purpose is demonstrated in Figure 4: the color change from blue to yellow occurred only when phosphate ion was added to the aqueous solution of the ensemble. Other anions failed to cause the color change.

In summary, we have assembled a novel colorimetric sensor for phosphate anions by simply mixing H-bpmp, which can be prepared readily in high yield, zinc perchlorate, and pyrocatechol violet, a commercially available dye, in water of a neutral pH value in a 1:2:1 molar ratio. The probe can detect phosphate ions in an aqueous solution of physiological pH, spectrophotometrically and visually, with unprecedented high selectivity towards phosphate anions over a variety of mono- and dianions. This sensor also allows a quantitative assay of the analyte in a neutral aqueous solution containing the ions that are commonly found in biological systems down to the concentration range of 10^{-5} M.[14] We are currently exploring the development of an inexpensive phosphate ion assay device useful for biological samples.

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Pyrophosphate Detection in Water by Fluorescence Competition Assays: Inducing Selectivity through the Choice of the Indicator**

Luigi Fabbrizzi,* Nathalie Marcotte,
Floriana Stomeo, and Angelo Taglietti

Fluorescent sensors have been designed for a variety of analytes during the last decade by following the classical "fluorophore-spacer-receptor" (FSR) approach in which a light-emitting fragment is covalently linked to a receptor subunit.[1] More recently, a different approach has been introduced, the "chemosensing ensemble" (CE) paradigm, which relies on the use of an indicator (**I**) bound to a receptor (**R**) by means of noncovalent interactions.[2] In this approach the highly colored or fluorescent probe **I** is displaced from **R** by the competing analyte (**S**), and this displacement produces a drastic change in the optical properties of released **I**. All the CE systems described until now were designed for anion sensing and most of them were based on hydrogen-bonding interactions. These interactions are relatively weak and in most cases do not compensate for the rather endothermic desolvation of **R** and **S**, which prevents the utilization of such receptors in pure water. We have recently reported the first example of a CE operating through metal–ligand interactions in which the receptor core is a dinuclear Cu^{II} macrobicyclic complex which is able to detect the carbonate ion in water through the revival of visible light emission of the displaced indicator.[3] The availability of optical molecular sensors for anion detection is highly beneficial for the investigation of aqueous media in food science, cell physiology, and environmental chemistry.[4]

Transition metal ions, for example, Cu^{II}, offer substantial advantages when designing CEs for anions. First of all, coordinatively unsaturated Cu^{II} complexes display strong binding tendencies towards anionic substrates because of the d^9 electronic configuration of the metal center, which ensures high ligand field stabilization effects: as a consequence, anionic substrates can be bound and effectively recognized even in the strongly solvating medium water. Moreover, the Cu^{II} ion provides an operative control of the signal as it is able to completely quench the emission of a coordinated indicator, either of an electron-transfer or an energy-transfer nature, by means of very efficient intramolecular processes.[5] We now demonstrate how the judicious choice of the indicator can turn macrocyclic complexes of Cu^{II} ions with well-known recognition tendencies towards anions into smart optical sensing devices, whose spectral and

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[*] Prof. L. Fabbrizzi, Dr. N. Marcotte, Dr. F. Stomeo, Dr. A. Taglietti
Dipartimento di Chimica Generale
Università di Pavia
viale Taramelli 12, 27100 Pavia (Italy)
Fax: (+39) 0382-528-544
E-mail: luigi.fabbrizzi@unipv.it

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