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Determination of Inorganic Phosphate in Serum and Saliva Using a Synthetic Receptor

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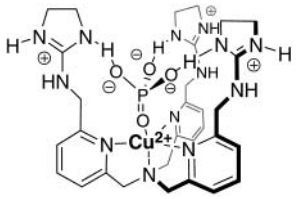
ABSTRACT



A C_{3v} symmetric synthetic receptor (1) was employed in an indicator-displacement assay to determine the phosphate concentrations in both horse serum and human saliva at biological pH. The determination of the phosphate concentrations in the serum and saliva using the colorimetric assay were 1.6 and 5.1 mM, respectively. These results further accentuate the usefulness of synthetic receptors in truly practical applications.

Oxyanions such as phosphate and sulfate are inorganic analytes commonly found in biological samples, beverages, and wastewater. In a clinical setting, phosphate levels in serum are determined as part of a routine blood analysis. The typical phosphate concentrations in adult human serum range from 0.81 to 1.45 mM.[1] Individuals with abnormally high phosphate levels are diagnosed with hyperphosphatemia, which manifests in acute or chronic renal failure. Those with low phosphate levels suffer from hypophosphatemia, which can be associated with rickets, hyperthyroidism, or Fanoci Syndrome. Phosphate is also prevalent in saliva, and the concentrations are variable, ranging from 5 to 14 mM.[2,3] The presence of phosphate ions in saliva serves to buffer the fluids within the oral cavity as bacterial acids are present.[4] This facilitates the repair of enamel and dentine, thereby maintaining the health and integrity of the teeth. Further, there is current interest by the NIH for developing sensing methods for saliva and making correlations to serum.[5]

There are several methods[6] reported in the literature for the determination for phosphate concentrations in blood; however, the methods most often used involve an ammonium molybdate reagent.[7] A typical serum sample is combined with an ammonium molybdate reagent under acidic conditions, and the inorganic phosphorus present reacts to form a phosphomolybdate complex. The absorbance of this complex at 340 nm is proportional to the phosphate concentration in the serum. Alternatively, the phosphomolybdate complex can be reduced to produce a color change, the absorbance of which is also proportional to the phosphate concentration in serum. Phosphate determinations in saliva are not as routine, but they are determined using the

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ammonium molybdate reagent,[2,8] as well as ion-selective electrode methods.[2,3]

The approach reported herein sought to quantify phosphate concentrations using a synthetic receptor, giving rise to a chemosensor. Our goal was not to improve upon the well-established inorganic assay, but rather to highlight the utility of synthetic receptors to analyze authentic samples akin to those found in a biomedical setting.

A chemosensor[9] often includes a molecular scaffold with a binding site and a signaling site. The binding of a guest "communicates" with the signaling site to generate an optical signal by which to observe the binding event. There is significant interest in developing synthetic receptors[10] and chemosensors[11] for binding phosphate in medicinal and biological applications.

The chemosensor used for this study takes advantage of an indicator-displacement assay comprised of metallo-host **1** and 5(and 6)-carboxyfluorescein. Host **1** was recently reported to have high affinity ($K_a = 1.5 \times 10^4$ M^{-1}) and excellent selectivity for phosphate in water at pH 7.4, where arsenate was the only other anion found to have a significant affinity.[12] The cavity of **1** was designed for the purpose of binding tetrahedral oxyanions with a scaffold derived from a trispyridyl subunit preorganized around a central Cu^{II} ion.[13] The periphery of the cavity is functionalized with guanidinium groups which are positioned to compliment the oxygens of a tetrahedral oxy-anion. The high selectivity of **1** for phosphate over other anions present in blood and saliva gave us a good opportunity for a colorimetric sensing ensemble,[14] a strategy that has been successful in our previous research endeavors.[15]

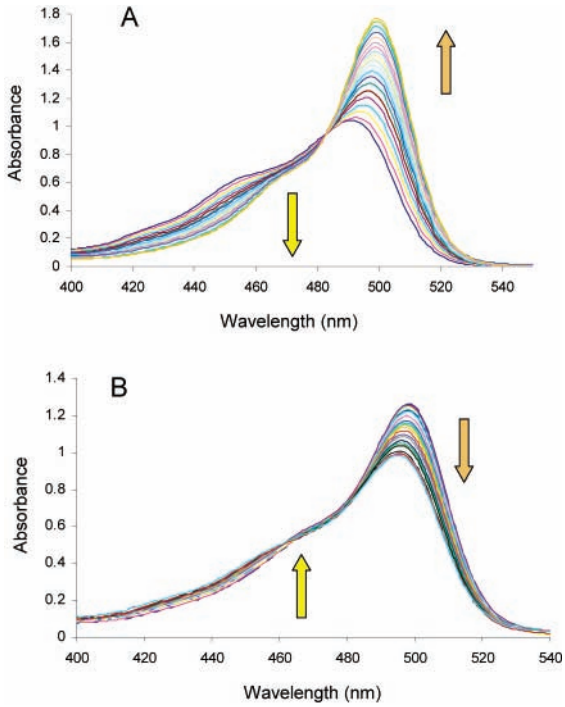
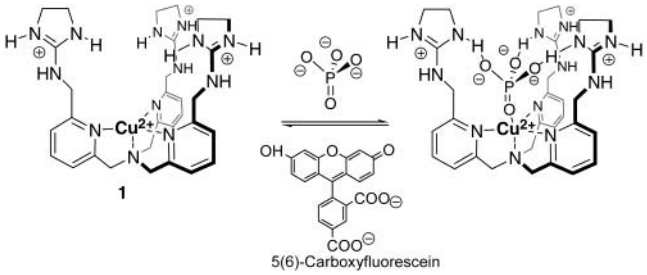


Figure 1. (A) UV/vis spectra of **1** binding to 5(and 6)-carboxyfluorescein in a 50:50 (v/v) water/methanol solution buffered at pH 7.4. The color changes from yellow to orange. (B) UV/vis spectra of the addition of aliquots of a phosphate solution (0.48 mM) to a 1:1 complex of **1** and dye (0.024 mM). The color changes from orange to yellow.

The sensing ensemble used for the studies reported herein was comprised of **1** and the indicator 5-(and 6)-carboxyfluorescein. This indicator is yellow in a 50:50 (v/v) water/methanol solution buffered at pH 7.4 with TRIS buffer. Upon addition of aliquots of **1** (as the chloride salt) to a solution of 5-(and 6)-carboxyflourescein, binding proceeds in a 1:1 stoichiometry with a color change from yellow to light orange (Figure 1A). This colorimetric response derives from a change in the microenvironment of the dye as it binds to **1**.



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As aliquots of a phosphate solution (as the sodium salt) are introduced to the host−dye complex, the light orange color reverts to the yellow color of free dye in solution (Figure 1B). This indicates that the phosphate displaces the dye from the host cavity, thereby creating a sensing ensemble for phosphate. This signaling motif is advantageous over the traditional methods of covalently attaching a signaling unit directly to the host scaffold in that no extra synthetic steps are necessary and that the signaling unit does not occupy a position on the scaffold that could potentially serve as an additional binding site for the guest.

The dye-displacement assay was used to generate a calibration curve for phosphate using UV/vis spectroscopy. Protein-free samples[16] of horse serum and saliva were analyzed using this sensing ensemble and compared to the calibration curve ($\lambda_{max} = 500$ nm). The phosphate concentration in the serum was determined to be 1.6 ($\pm$0.2)[17] mM and that of the human saliva was 5.5 ($\pm$0.5)[18] mM. The phosphate concentrations in the same samples were measured using a commercially available inorganic phosphorus kit from Diagnostic Chemicals Limited, which yielded comparable values of 1.8 ($\pm$ 0.2)[17] mM and 5.1 ($\pm$0.6)[17] mM for serum and saliva, respectively. The literature values compare well, with horse serum ranging from 1.3 to 1.7 mM[19] and human saliva ranging from 5 to 14 mM.

In summary, metallo-receptor **1** when combined with 5(and 6)-carboxyfluorescein makes an effective chemosensor for inorganic phosphate in complex biological fluids. The concentration of phosphate in both serum and saliva is high; therefore, a more sensitive technique (e.g., fluorescence) is not necessary. The results of the assay are comparable with clinically approved methods of phosphate determination. The success of using a synthetic receptor and an indicator-displacement approach for a medicinal application highlights the increasing utility of synthetic systems in truly practical applications.

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(16) Ultracentrifugation was used to remove the native proteins from the samples. The assay does not work with the proteins present as the solution becomes occluded and the absorbance spectrum altered.

(17) Errors reported as the standard deviation of six determinations of phosphate concentration.

(18) Errors reported as the standard deviation of three determinations of phosphate concentration.

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