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Colorimetric Sensor for ATP in Aqueous Solution

D. Amilan Jose, Sandhya Mishra, Amrita Ghosh, Anupama Shrivastav, Sanjiv K. Mishra, and Amitava Das*

Central Salt and Marine Chemicals Research Institute, (CSIR) Bhavnagar 364002, Gujarat, India

amitava@csmcri.org

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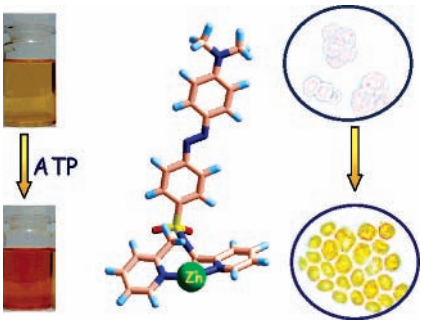
ABSTRACT



A new chromogenic complex 1·Zn has been synthesized, and its interactions with different biologically important phosphates have been investigated in aqueous solution (pH ∼ 7.2). A visual color change can be detected on binding of ATP to 1·Zn, whereas no such change is observed when other biologically related anions (AMP, ADP, PPi, or Phosphate) are used. Complex 1·Zn can also be used as a staining agent for yeast cells allowing detection under normal light microscopy.

The design of a chemosensor for selective recognition and sensing of a particular anion is an area of immense importance.[1,2] In this context, sensors that can detect and sense biologically important anions in aqueous environments and under physiological pH are of special significance owing to their potential applications as biological markers.[3] Among various anions, recognition of phosphates, pyrophosphate, nucleotides, and nucleosides is crucial in this regard.[4,5] Among many phosphate anions, adenosine triphosphate (ATP) is a multifunctional nucleotide that is most important as a *molecular currency* of intracellular energy transfer.[6] In this role, ATP transports chemical energy within cells for metabolism. It is produced as an energy source during the processes of photosynthesis and cellular respiration and is consumed by many enzymes and a multitude of cellular processes, including biosynthetic reactions, motility, and cell

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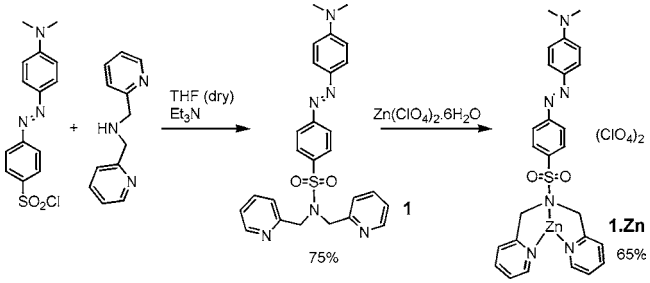
division. ATP is also involved in DNA duplication and transcription.[7] Thus, selective recognition of ATP in aqueous media has emerged as an important area of research. However, existing reports on the recognition of ATP are mostly based on changes in fluorescence or electrochemical properties.[8,9] Examples for the colorimetric detection of ATP in aqueous environments are rare.[10]

Herein, we report a new chromogenic complex **1·Zn**, which can be used to bind ATP in aqueous solutions at physiological pH. The zinc dipicolylamine unit in **1·Zn** acts as the receptor fragment for ATP, whereas the dimethylamino-phenylazo group acts as the signaling group for reporting the binding, inducing a visibly detectable color change. This complex can also be used for staining yeast cells, and stained cells can be viewed by simple light microscopy.

4-(4-Dimethylamino-phenylazo)-*N*,*N*-bispyridin-2-ylmethyl-benzenesulfonamide (**1**) was synthesized by a one-step reaction (Scheme 1) and isolated in pure form. A methanolic

Scheme 1. Synthetic Methodology of Complex **1·Zn**



solution of **1** was reacted with an aqueous solution of $Zn(ClO_4)_2$ to yield the receptor molecule **1·Zn** at room temperature (Scheme 1). Both **1** and **1·Zn** were characterized by standard analytical and spectroscopic techniques.[11]

Electronic spectra for a 50 μM solution of **1·Zn** in the absence and presence of various anions were recorded in acetonitrile solutions (Supporting Information). The absorption spectrum recorded for **1·Zn** showed an intense absorption band at 439 nm ($\epsilon = 15\,720\ M^{-1}\ cm^{-1}$). On addition of excess tetrabutyl ammonium phosphate (TBAP), a new absorption band appeared at 510 nm ($\epsilon = 19\,880\ M^{-1}\ cm^{-1}$) (Figure 1). However, no such spectral change could be

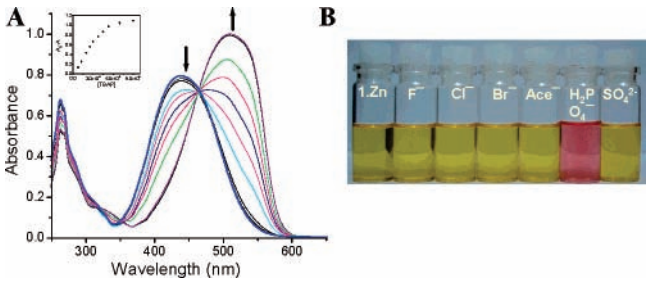


Figure 1. (A) Change in absorption spectra of **1·Zn** (50 μM) at 25 °C in acetonitrile in the presence of varying [TBAP] (5−200 μM). Inset: Job plot for the spectrophotometric titration. (B) Change in color of **1·Zn** in acetonitrile in the presence of different anions (75 μM). From left to right: blank, F^-, Cl^-, Br^-, CH_3COO^-, $H_2PO_4^-$, and SO_4^{2-}.

observed on addition of an excess of other anions such as F^-, Br^-, Cl^-, I^-, CH_3COO^-, HSO_4^-, or $H_2SO_4^-$ (Supporting Information). A systematic change in the spectral pattern for **1·Zn**, associated with varying [TBAP], is shown in Figure 1A.

The presence of an isosbestic point at 454 nm signified the presence of an equilibrium process. No further spectral change was registered when TBAP was added beyond the mole ratio of 1.2 equiv. The change in color on binding of a phosphate ion to the Zn(II) center in **1·Zn** was associated with the perturbation of the energy of the frontier orbitals of the donor amine functionality and the acceptor azo fragment. Binding of **1** (λ_{max} = 423 nm) to Zn(II) in CH_3CN caused a shift in the maxima (λ_{max} = 439 nm) of the charge-transfer spectra, whereas binding of the anionic phosphate to the Zn(II) center in **1·Zn** has caused a further red shift. A similar shift is also reported for other sensor molecules, where the signalling unit is separated from the receptor unit by the $-SO_2-$ functionality.[12] This study revealed that **1·Zn** behaved as a specific colorimetric sensor

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(11) Compound **1** (75%): ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.38−8.36 (Ar−H, 2H, d, J = 4.4 Hz), 7.93−7.88 (Ar-H, 6H, m), 7.58−7.51 (Ar-H, 2H, t, J = 7.6 Hz), 7.35−7.26 (Ar−H, 2H, m), 7.11−7.05 (Ar−H, 2H, t, J = 7.1 Hz), 6.79−6.75 (Ar−H, 2H, d, J = 8.8 Hz), 4.63 ($-CH_2$, s, 4H), 3.13($-CH_3$, s, 6H); LC-Mass (+ mode) m/z 488.15 (M^++ 1). Elemental analysis calcd: C, 64.18; H, 5.39; N, 17.27; S, 6.59. Exptl: C, 64.3; H, 5.4; N, 17.1; S, 6.7. Compound **1·Zn** (65%): ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.85−8.82 (Ar−H, 2H, d, J = 5.2 Hz), 8.25−8.19 (Ar−H, 2H, t, J = 6.8 Hz), 7.94−7.87 (Ar−H, 6H, m), 7.79−7.66 (Ar−H, 4H, m), 6.90−6.86 (Ar−H, 2H, d, J = 8.8 Hz), 4.95 ($-CH_2$, s, 4H), 3.15 ($-CH_3$, s, 6H); LC-Mass (+ mode) m/z 567.27($M^+\cdot H_2O$ + 1). Elemental analysis calcd: C, 56.57; H, 4.75; N, 15.23; S, 5.81. Exptl: C, 56.8; H, 4.7; N, 15.1; S, 5.7.

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for $H_2PO_4^-$ in acetonitrile solutions. A Job's plot confirms a 1:1 binding of **1·Zn** and $H_2PO_4^-$, and binding constants ([5.62 ± 0.08] × 10^5 M^{-1}) were evaluated from the absorption titration profile (inset in Figure 1). Binding of the phosphate ion to **1·Zn** was also confirmed by ^{31}P NMR spectroscopy in CH_3CN. A signal corresponding to the ^{31}P response in the phosphate ion appeared at −31.5 ppm, which was found to be shifted to −32.7 ppm (Supporting Information) upon addition of 1.2 mol equiv of **1·Zn**. This upfield shift indicated efficient binding of the phosphate ion to the Zn(II) center. The selectivity of the receptor molecule, **1·Zn**, toward the phosphate ion over other anions prompted us to investigate the binding of **1·Zn** toward different biologically important anions such as ATP, ADP (adenosine diphosphate), AMP (adenosine monophosphate), and PPi (pyrophosphate) in aqueous media.

The ground-state absorption spectra of **1·Zn** (25 μM) were recorded in a buffer solution [10 mM HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), pH ∼ 7.2] at 25 °C. An intense absorption band centered at 463 nm was observed. On addition of an aqueous solution of ATP, this absorption maximum was found to be shifted at 484 nm. An associated color change from pale yellow to light pink was observed (Figure 2). However, upon addition of

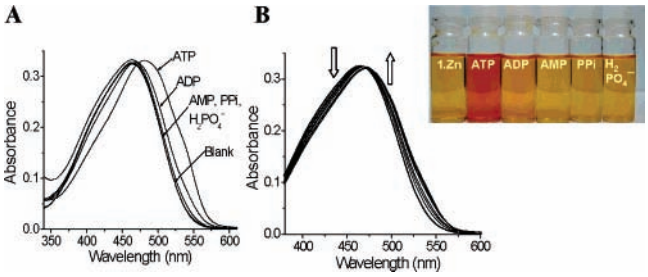

Figure 2. (A) Absorbance spectra of **1·Zn** (25 μM) in HEPES buffer solution (pH ∼ 7.2) at 25 °C in the presence of various anions (260 μM) and (B) the changes in the spectral pattern in the presence of varying [ATP] (2−260 μM). Inset: change in color of **1·Zn** in aqueous solution. From left to right: blank, with ATP, ADP, AMP, PPi, and $H_2PO_4^-$.

ADP to a **1·Zn** solution, a much smaller red shift (8 nm) in λ_{max} occurred and no change in color could be registered by the naked eye. Furthermore, no change in absorption spectra was observed on addition of AMP, PPi, or $H_2PO_4^-$ (Figure 2A). A similar experiment with CTP gave a less appreciable red shift than that observed for ATP (Supporting Information). The respective binding constants for ATP, CTP, and ADP, evaluated from spectral titration profiles, were found to be 1130 ± 6, 772 ± 5, and 250 ± 6 M^{-1} in aqueous solution (pH ∼ 7.2) at 25 °C. However, this was not possible for AMP and the other anions, as the associated spectral changes were negligible due to much weaker binding.

Receptor molecule **1·Zn** showed a weak luminescence on excitation at 463 nm. The weak emission was found to be partially quenched on addition of ATP, whereas no such decrease in emission intensity was observed when excess ADP, AMP, PPi, or $H_2PO_4^-$ was added to the HEPES buffer solution (Supporting Information). This study further confirmed the efficient and selective binding of ATP to **1·Zn**. Binding of ATP was also confirmed by ^{31}P NMR spectroscopy (Figure 3). A downfield shift for the ^{31}P signal for the

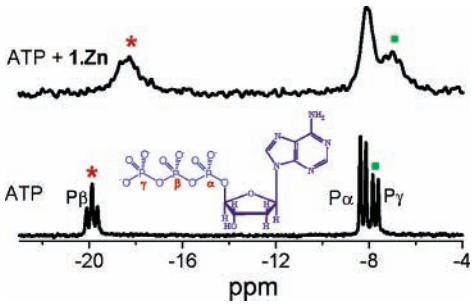

Figure 3. Partial ^{31}P NMR spectrum of ATP alone and ATP + **1·Zn** recorded in D_2O at room temperature.

P-atoms of the ATP bound to **1·Zn** was observed. A similar shift has been reported by others.[13] As expected, the shift was more prominent for the γ and β P-atom of the ATP, whereas the shift for the α P-atom was less. A very insignificant shift in ^{31}P signals was observed when similar experiments were repeated for ADP, and no such shift was observed when identical experiments were performed with AMP (Supporting Information).

Thus, the ^{31}P NMR spectral data also confirm the observed trend in binding affinity (ATP > ADP ≫ AMP). Presumably, the enhanced electrostatic interaction between ATP and **1·Zn** is crucial for efficient **1·Zn**−O (phosphate) binding. Hence, the observed binding preference of **1·Zn**, ATP > ADP ≫ AMP, could be attributed to the difference in the number of the anionic charges of the phosphate species. Among ATP and CTP, the presence of the pyrimidone functionality in CTP has made it a weaker electron donor, and this presumably is reflected in the lower affinity constant. However, being a triphosphate, it binds more efficiently to **1·Zn**, as compared to ADP and AMP. Higher selectivity toward ATP and the visual change in color on binding led us to consider the possible use of this reagent for the staining of live yeast cells.

Saccharomyces cerevisiae (*S. cerevisiae*), a eukaryotic microbe, derive their energy in the form of ATP,[14] and the surface of these cells possesses negatively charged ATP ions. We have, therefore, been able to use the receptor molecule **1·Zn** for staining eukaryotic cells. The images of the

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colorless yeast cells, viewed under normal light microscopy (AXIO IMAGER, Carl Zeiss), are shown in Figure 4A. These

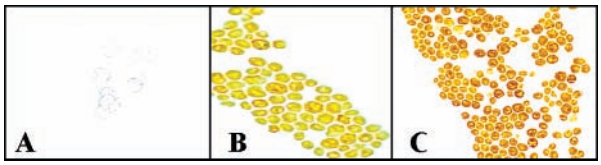

Figure 4. Light microscopy images (100 ×) of (A) blank yeast cells, (B) yeast cells with **1·Zn**, and (C) after washing the stained cells with a water/ethanol (70:30, v/v) mixture.

cells were exposed to the receptor molecule **1·Zn** for ~20 min and were then viewed under a light microscope. Images of the treated cells thus observed are shown in Figure 4B (with similar magnification). Figure 4 revealed that treated cells became stained with **1·Zn** and that the color of the cells changed to reddish yellow (Figure 4B). The change in color of yeast cells indicated that the negatively charged phosphate groups on the surface of the cells were effectively bound to the receptor molecule **1·Zn**. The staining was found to be stable even after subsequent washing with water/ethanol (70:30, v/v) mixtures (Figure 4C). Ethanol is known to act as a dehydrator and as a good fixative of biological samples. Besides cleaning the residual or extraneous (unbound) dye present near the stained sample, **1·Zn** also denatures the protein and exposes the reactive groups allowing attachment to the dye giving a more intense color. Control experiments were performed by treating these cells with **1** or a solution of free Zn(II) ions, and no such change in color was observed (Supporting Information). This confirms the binding of the ATP ions of the cell surface to **1·Zn** as the plausible reason for the observed staining.

SEM images of blank and stained yeast cells have been recorded (Figure 5) to reveal the change in the morphology of the outer surface of the yeast cells on staining, as a

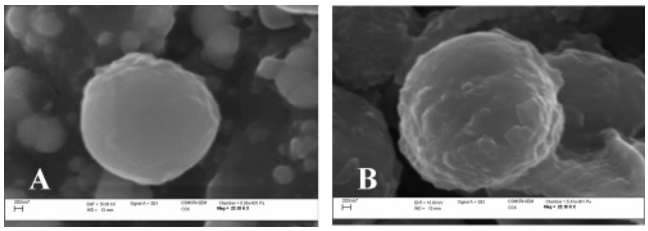

Figure 5. SEM images of (A) blank yeast cells and (B) with **1·Zn**.

maximum concentration of ATP is released through mitochondria in the periplasmic space (Supporting Information).[15] Several ATP binding cassette proteins are also reported to accumulate in the plasma membrane (space between the cell wall and the cell membrane).[16,17] SEM images of yeast cells without dye were found to be smooth in contrast to the images of the yeast cells with dye. Images of the stained cell surfaces were found to be rough, showing the presence of the extraneous material, i.e., **1·Zn**, adhering to the cells. Thus, SEM images clearly demonstrated a distinctive change in the outer surface of the individual stained cells. Thus, SEM images also confirmed the binding of the receptor molecule **1·Zn** to the ATP localized on the outer membrane of the yeast cells and therefore could be used as a colorimetric organelle probe. Structural evidence for the binding of the ATP to the Zn(II) center in the protein structure has been provided very recently by Sharma and co-workers.[18]

B. D. Smith and co-workers have recently demonstrated the ability of fluorescently labeled bis-Zn(II)-dipicolylamine-based complexes to stain the surface of bacterial cells using fluorescent microscopy techniques, and it was found to be more specific to PPi than ATP.[19] In this article, we have successfully demonstrated that chromogenic metal complex **1·Zn** selectively binds to ATP in contrast to other biologically important anions such as ADP, AMP, PPi, or phosphate under aqueous conditions. Furthermore, this receptor molecule can be used as a colorimetric staining agent for yeast (*S. cerevisiae*) cells, and staining can be viewed by simple light microscopy. The selectivity of **1·Zn** toward ATP offers us the opportunity of using this as a yeast viability stain.

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Supporting Information Available: Syntheses of **1** and **1·Zn**; sample preparation; emission spectra of **1·Zn** in the presence of different anions and AXP (X = T, D, M); UV−vis spectra of **1** and **1·Zn** in the presence of various anions; Benesi−Hildebrand plot; change in ^{31}P NMR of TBAP with **1·Zn**; changes in ^{31}P NMR of ADP with **1·Zn**; light microscope and SEM images. This material is available free of charge via the Internet at http://pubs.acs.org.

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