Prayogik Rasayan

Colorimetric Detection of Histidine and Histidine-Rich Proteins at Physiological pH via Analyte-Driven Ni^{2+} Sequestration

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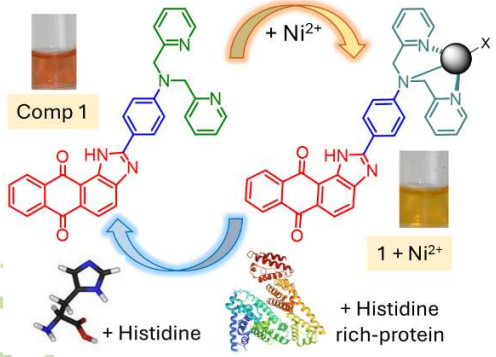
Abstract

An anthraimidazoldione-based chromogenic probe is synthesized that exhibits dual absorption bands in pH 7.4 PBS buffer due to n→π* transitions and intramolecular charge transfer (ICT). Coordination with Ni^{2+} causes a blue shift in the ICT band (492 nm → 410 nm) and changes in solution color from red to yellow, indicating strong interaction with a 1:1 binding stoichiometry. Further, the insitu formed Ni^{2+} complex shows highly selective ratiometric response towards histidine, with a detection limit of 12 μM. Histidine addition restores the ICT band at 502 nm with restoration of original red color of native probe. Reversible interaction with histidine confirms the mechanism of Ni^{2+} sequestration. Comparative studies with Cu^{2+} revealed similar histidine-induced absorbance restoration, but in lesser extent due to lower binding affinity. Moreover, the Ni-complex can successfully differentiate proteins based on numbers of their histidine residues.



Keywords: Anthraimidazoldione; Intramolecular Charge-transfer; Colorimetric response; Histidine; Ni^{2+}- sequestration

1. Introduction

Histidine, an essential amino acid with imidazole side chain, which serves as a critical mediator in immune responses, gastric acid secretion, and neurotransmission.[1] Additionally, histidine contributes to the biosynthesis of hemoglobin and myoglobin and in maintaining acid-base balance. In addition, histidine often resides at the active sites of enzymes, facilitating proton transfer and catalysis.[2] It also aids in metal ion coordination, binding essential metals like zinc, copper, and nickel, crucial for various metalloproteins and enzymes.[3] Furthermore, the antioxidant properties of histidine help neutralize free radicals, reducing oxidative stress and protecting cellular components from damage.[4] Despite these, abnormal histidine levels are linked to various diseases. Elevated level of histidine can cause impaired metabolism and neurological disorders, while its low level can cause rheumatoid arthritis, anaemia and chronic kidney disease etc.[5] Thus, monitoring histidine levels is essential, especially in populations at risk for metabolic disorders, kidney dysfunction, or inflammatory diseases, to mitigate these potential effects.[6]

Considering these, over the years various optical probes have been developed for quantitative probing of histidine.[7] However, most of these probes suffer from poor sensitivity in the aqueous medium, cross-reactivity and lack of perceptible colorimetric response. Over the years, various metal (Cu, Ni, Zn) complex based optical probes have been utilized for detection of histidine in the aqueous medium.[8] However, in many cases, significant interference could be seen from other amino acids, such as cysteine, and homocysteine, which indicated possibility of receiving false positive signal during real-life sample analysis. Also, most of these reported sensors exerted turn-on fluorescence response in presence of histidine without perceptible changes in color. For such kind of sensors, using spectrofluorimeter is essential for visualization and quantification purposes.

Considering these, herein we have utilized an easily scalable anthraimidazoldione based charge transfer probe and utilize the in-situ formed Nickel complex for sensing of histidine at biological pH. The addition of histidine could immediately result changes in solution from yellow to red. Such distinct color difference enables detection of histidine without engaging any sophisticated instrument. Along with Nickel, we have also involved insitu formed copper complex for screening of histidine. Not only we observed a significant interference from cysteine, but also the response towards histidine was found to be compromised than that witnessed with Ni2+. In addition, we also checked interaction with proteins containing different numbers of histidine residues, such as HSA and lysozyme. As expected, protein with higher number of histidine residue (HSA) could render larger change in optical signal.

2. Experimental

Materials and methods: The chemicals (solvents, precursors and reagents) were bought from best-known local supplier and used without further purification. Bruker Advance DRX 400 spectrometers running at 400 and 100 MHz was used for ^{1}H and ^{13}C NMR spectroscopy respectively. The Perkin Elmer FT-IR spectrometer BX for recording the IR spectrum. Mass spectrum was recorded on a Micromass Q-TOF Micro TM spectrometer.

Spectroscopic Studies: A Shimadzu model 2100 spectrometer and a Cary Eclipse spectrofluorimeter, respectively, were used to capture the UV-visible spectra. The stock solution of 1 was prepared in DMSO (1 mM) and diluted with pH 7.4 PBS buffer (0.05 M) to maintain the desired concentration (10 µM) for spectroscopic studies. The final concentration of DMSO did not exceed more than 1% in final solution. Stock solutions of both metal ions and amino acids (50 mM) were freshly prepared in water before use.

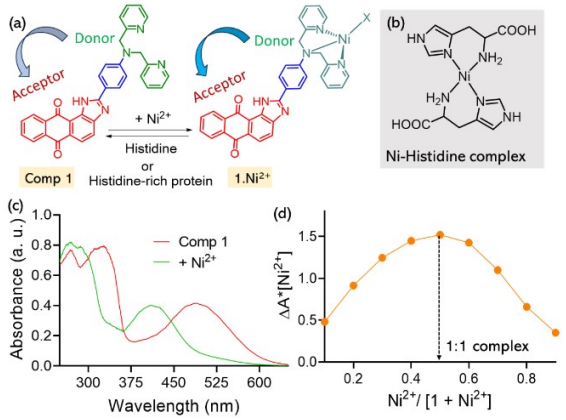


Figure 1. (a) Schematic diagram shows Interaction of **1**.Ni^{2+} with histidine. (b) Possible structure of Ni-histidine complex (c) UV-visible spectra of compound **1** (10 µM) with and without Ni^{2+} (0.5 mM) in pH 7.4 PBS buffer medium. (d) Job's plot analysis shows 1:1 binding interaction between **1** and Ni^{2+}.

3. Result and discussion:

Design and Synthesis of Charge-transfer Probe: Compound 1 was synthesized by reacting p-BPA benzaldehyde with 1,2-diaminoanthraquinone under reflux conditions.[9] The compound was thoroughly characterized using FT-IR, mass spectrometry, and ^1H and ^{13}C NMR techniques. Literature reports indicate two distinct protonation pKa values for the bispicolyl (BPA) unit: 3.6 for the pyridine nitrogen and 8.6 for the tertiary nitrogen. The UV-visible spectrum of Compound 1 in a pH 7.4 (0.05 M PBS) buffered medium exhibited two absorption maxima. The peak at 328 nm was attributed to the n→π* transition, while the lower-energy band around 492 nm was assigned to intramolecular charge transfer (ICT) interactions.[10] The ICT likely arises from electron donation by the electron-rich BPA unit to the electron-deficient anthraimidazoldione moiety. When the aqueous solution of Compound 1 was treated with Ni^{2+} (~0.5 mM), the ICT band exhibited a blue shift of 82 nm, resulting in a solution color change from red to yellow. This shift likely reflects Ni^{2+} coordination to the BPA moiety, which disrupts charge transfer efficiency. Concurrently, the band at 328 nm shifted to 280 nm, indicating the involvement of nitrogen lone pairs in Ni^{2+} binding.[11] Job's plot analysis confirmed a 1:1 binding stoichiometry between Compound 1 and Ni^{2+} in aqueous solution.

Interaction with Histidine and histidine-rich proteins: The behavior of the Ni^{2+} complex of Compound 1 was further investigated against a range of amino acids. Among the tested essential amino acids, only histidine induced a color change from yellow to red, restoring the broad absorption band centered around 502 nm. UV-visible titration studies with histidine revealed an increase in absorbance at 502 nm and a concurrent hypochromic shift at 408 nm. When the changes in absorbance at 502 and 408 nm were plotted against histidine concentration, a linear regression equation (Y = 0.223 + 1.51X) was obtained, with a coefficient of determination (R^2) of ~0.99. This ratiometric response is advantageous for real-life sample analysis, improving signal-to-noise ratios by mitigating background interference. Furthermore, titration studies indicated a limit of detection (LOD) as low as 12 µM for histidine. Selectivity tests revealed that no other amino acids induced significant absorbance changes, underscoring the system's high specificity for histidine. Given that Compound 1 is also known to interact with metal ions such as Cu^{2+}, its ability to detect histidine in the presence of Cu^{2+} was evaluated. Like Ni^{2+}, the addition of histidine to the 1.Cu^{2+} complex induced a color change from yellow to red, accompanied by an increase in the absorption band at 502 nm. However, the absorbance change was ~4.5-fold with the 1.Ni^{2+} complex compared to ~2.9-fold with 1.Cu^{2+}, likely due to the higher binding affinity of Ni^{2+} for histidine.[12] The system was also tested for its ability to detect histidine in histidine-rich proteins. Upon addition of human serum albumin (HSA) and lysozyme, significant signal recovery (~87%) was observed with HSA, while only ~8% was seen with lysozyme. This difference can be attributed to the number of histidine residues, as HSA contains 16 histidine residues compared to just one in lysozyme.[13]

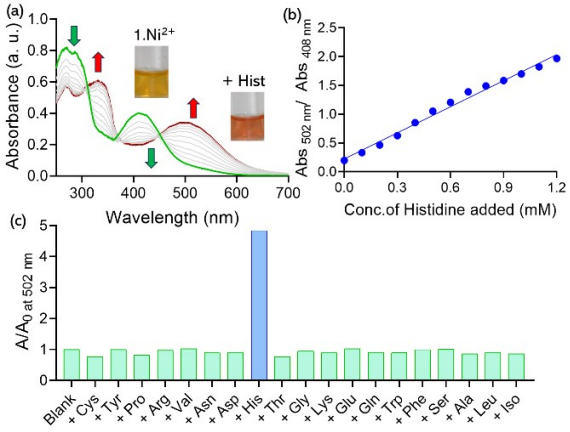


Figure 2. (a) UV-visible titration of **1**.Ni^{2+} (10 µM, 1:50) with histidine (0- 1 mM) in pH 7.4 PBS buffer medium. (b) Changes in absorbance ratios of **1**.Ni^{2+} (10 µM, 1:50) at 502 and 408 nm bands upon addition of histidine. (c) Changes in absorbance of **1**.Ni^{2+} (10 µM, 1:50) at 502 nm upon addition of various amino acids (1 mM) in pH 7.4 PBS buffer medium.

Mechanistic Investigation: To understand the mechanism of interaction between 1.Ni^{2+} and histidine, further spectroscopic studies were conducted. The UV-visible spectrum of the 1.Ni^{2+} complex in the presence of ~1 mM histidine closely resembled that of the free probe, indicating that histidine releases the free probe by sequestering Ni^{2+}. Recovery experiments, in which the solution was alternately spiked with Ni^{2+} (0.5 mM) and histidine (1 mM), revealed reversible absorbance changes at 502 nm over 3–4 cycles. The absorbance at 502 nm was substantially reduced (< 0.1) in the presence of Ni^{2+} but increased significantly (> 0.3) upon histidine addition. This reversibility indicates that histidine preferentially binds Ni^{2+}, preventing ternary complex formation.[14] Moreover, the reusability of the probe solution

for multiple detection cycles highlights its potential for cost-effective analysis.

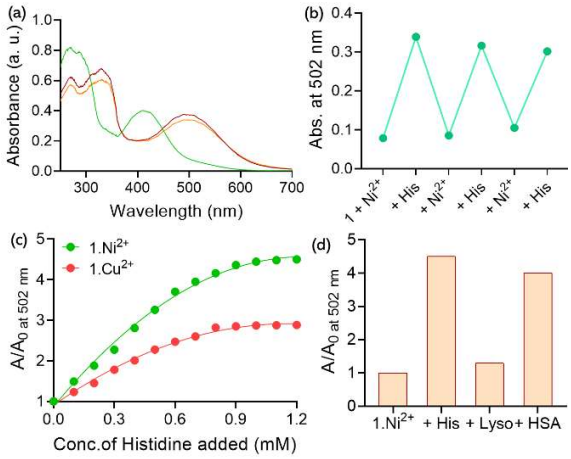


Figure 3. (a) UV-visible spectra of 1 and **1**.Ni^{2+} (10 μM, 1:50) with histidine (1 mM) in pH 7.4 PBS buffer medium. (b) Changes in absorbance upon sequential addition of Ni^{2+} (0.5 mM) and histidine (1 mM) at pH 7.4 in PBS buffer. (c) Changes in absorbance of 1.Ni^{2+} and 1.Cu^{2+} at 502 nm upon addition of histidine (0 – 1.2 mM) at pH 7.4 in PBS buffer. (d) Changes in absorbance of 1.Ni^{2+} (10 μM, 1:50) at 502 nm upon addition of HSA and lysozyme at pH 7.4 in PBS buffer

4. Conclusions

In conclusion, we have developed an anthraimidazoldione based chromogenic probe (**1**) with metal ion chelating bispicolyl unit at the terminal position. At pH 7.4 buffer, compound exhibited bright red color owing to intramolecular charge transfer (ICT) interactions. Upon coordination with Ni^{2+} ions, a significant blue shift in the ICT band (492 nm → 410 nm) was observed along with change in solution color from red to yellow. The in-situ prepared Ni^{2+} complex demonstrated highly selective and sensitive detection of histidine among various amino acids, restoring the ICT band at 502 nm with a ratiometric response. Histidine addition also induced reversible spectral changes, supporting a mechanism involving the release of the free probe by sequestration of Ni^{2+}. This system could also differentiate proteins based on numbers of their histidine residues. For example, human serum albumin (HSA) with 16 histidine residues resulted in ~87% recovery optical signal. Additionally, comparative studies with the Cu^{2+} complex indicated a superior response to histidine with the Ni^{2+} system. Not only that, interference from cysteine was witnessed when insitu formed copper complex was utilized as a sensing probe. These findings highlight the utility a metal complex based chromogenic probe for reversible and ratiometric analysis of histidine in diverse matrices.

5. Acknowledgements

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6. Notes and References

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Dr. Nilanjan is currently working as an assistant professor at the Birla Institute of Technology and Science Pilani, Hyderabad campus. His laboratory mainly focuses on designing optical probes for biomolecule detection, drug delivery, and biomedical applications, while also addressing agricultural and wastewater management issues. He has published over 125 papers in international peer-reviewed journals (H index: 32). Dr. Nilanjan has received several awards, including the NASI-Young Scientist Platinum Jubilee Award and Thieme Chemistry Journals Award in 2022, the MSCA- Seal of Excellence Award from the European Commission in 2021, the JSPS Postdoctoral Fellowship from the Japan Society for the Promotion of Science and the DS Kothari Postdoctoral Fellowship from the MHRD-University Grant Commission in 2018. In 2024, he was elected as fellow of Indian Chemical Society, named as a Rising Star in Materials Science by ACS Materials Au and an Emerging Investigator by Organic Materials (Thieme). He was also ranked among the World's Top 2% Scientists both in 2023 and 2024 by Stanford University. Dr. Nilanjan also serves on the editorial boards of several journals, including Frontiers in Chemistry, Tetrahedron Green Chemistry (Elsevier) and Discover Molecules (Springer), Journal of Chemical Research (SAGE).