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Smart optical probe for 'equipment-free' detection of oxalate in biological fluids and plant-derived food items

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ABSTRACT

A reversible 'turn-on' sensor has been designed for 'naked-eye' detection of oxalate at nanomolar concentration (~12.5 nM) at pH 7.4. The sensory system shows a highly specific response towards oxalate among a wide range of antinutrients and biologically relevant anionic species. Mechanistic investigations indicate that oxalate can turn the pink-colored solution colorless by dissociating the preformed metal complex. Additionally, high specificity and good accuracy with recovery values ranging from 93.3 to 105.0% were obtained during oxalate estimation in spiked water and human urine samples, confirming the suitability of the present method in estimating trace-level of oxalate in complex matrices. With these results, quantitative estimations of endogenous oxalate were achieved in more than twenty-five different agricultural crops. Finally, low-cost, portable paper strips were developed for on-site detection of oxalate.

1. Introduction

'Chelating agents' like oxalate and phytate are considered as 'antinutrient' due to their strong binding affinity towards physiologically-abundant bivalent metal ions such as calcium, zinc, copper, and magnesium etc [1]. By forming insoluble salts, they can reduce the bioavailability of these metal ions and trigger health problems, like convulsions, irregularity in carbohydrate metabolism *etc* [2,3]. Considering the high abundance of oxalate in the wide range of agricultural crops, here we have focused on designing small molecular probe for rapid, on-site detection of oxalate without involving any equipment (not even a UV torch). In addition to these above-mentioned health problems, the accumulation of excess oxalate salts (insoluble) in kidney tubules causes renal failure, cardiomyopathy disorder, and the development of kidney stones *etc* [4]. Since there is no enzyme available in the human body for the degradation of oxalate, it is essential to control the amount of oxalate uptake, especially for the patients suffering from hyperoxaluria [5]. A literature survey indicates that the established analytical methods known for oxalate detection mostly involve advanced techniques, such as enzymatic assay, HPLC, Capillary electrophoresis, ion chromatography *etc*., which require complicated, multi-step sample preparation protocols and expensive equipment [6–10]. Though these methods show reasonably high efficiency in the determination of urinary oxalate level, they are mostly found incompetent in analyzing endogenous oxalate content in different agricultural crops. This is probably due to the interference caused by the carbohydrate derivates present in the plant tissues [11,12]. Additionally, the execution and maintenance of these instruments need a constant supply of energy and trained personnel. Though in general, colorimetric sensors for oxalate can address most of these issues, their numbers are surprisingly less in the literature. Among them, the majority of the sensing systems exploit indicator displacement assay (IDA) as their recognition strategy (Table S1) [13–21]. Since the IDA works on the multi-component systems, rigorous standardization procedures need to be followed for their optimization.

In continuation of our earlier investigations on biomolecular sensing [22–25], herein we have employed a simple 'catch-free' strategy or displacement approach for rapid, naked-eye detection of oxalate at a nanomolar concentration (~12.5 nM) in water (pH 7.4) (Fig. 1b). Unlike the multi-component based IDA assay, the optimization steps involved in the 'catch-free' method are generally less complicated and more time-saving. The rhodamine-based colorimetric probe engaged in this case showed selective

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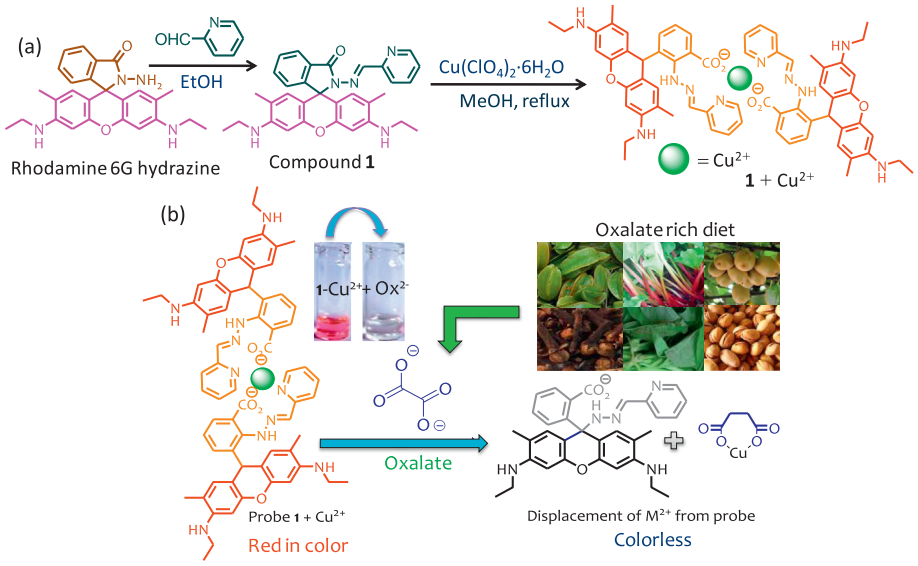

Fig. 1. (a) Synthetic route of compound **1** and **1**-Cu^{2+}. (b) The principle of oxalate sensing using in-situ generated **1**-Cu^{2+} complex (displacement approach).

response towards oxalate (a change in color from bright pink to colorless) only in presence of Cu^{2+} [26]. Rhodamine was chosen purposefully as the signaling moiety due to its easily modifiable synthetic backbone and excellent stimuli-responsive photophysical behavior [27–30]. Considering the high selectivity as well as the sensitivity of probe towards oxalate, the present system was further employed for the quantification of soluble oxalate (endogenous) contents in more than twenty-five different agriculture crops. In addition to this, the protocol was found to be equally effective in analyzing drinking water and human urine samples. Portable color strips were also developed for rapid, on-site detection of oxalate. As the present method does not involve any sophisticated instruments or complicated sample preparation steps, people with limited knowledge in science will be able to handle it.

2. Results and discussion

2.1. Photophysical properties of 1 + Cu^{2+}-complex at pH 7.4

UV–visible spectrum of **1** showed presence of two absorption bands at 247 nm ($\varepsilon = 2.42 \times 10^4$) and 310 nm ($\varepsilon = 2.03 \times 10^4$) (Fig. S1). The band at 247 nm could be assigned due to π- π* transition, while n-π* transition resulted in the formation of 310 nm band [31]. The presence of orthogonal spirolactam ring in **1** prevented electronic mixing of the electron-rich signaling moiety (xanthene) with the comparatively electron deficient recognition (pyridine end) site. Therefore, in the free probe, HOMO was found to be largely concentrated on the xanthene fragment whereas, LUMO mainly focused on the pyridine unit (Fig. S2) [32–34]. The presence of $Cu(NO_3)_2$ induced a distinct change in color of **1** from colorless to bright pink with the appearance of a new absorption band at 528 nm in the visible region. This major red-shift in the n-π* transition band (~240 nm) was originated from the formation of the extended conjugated structure followed by reversible spirolactam ring opening. The increase in the effective conjugation was further evidenced by the non-localized distribution of frontier molecular orbitals (FMOs) over the entire molecular framework (Fig. S3). Along with this, shortening of N–C_1 bond length (1.48 A° to 1.35 A°) upon metal ion complexation also suggested the partial double bond characteristics of this bond due to electronic push from peripheral nitrogen ends (Fig. 2c) [35,36]. As compound **1** showed a 1:2 complexation with Cu^{2+} ion (the detail investigation has been reported earlier, please see reference [26]), we have synthesized the copper complex with **1** keeping their molar ratio fixed at 2:1 (**1**: Cu^{2+} ion = 2:1) (Fig. 1a) [26].

2.2. Colorimetric sensing of oxalate using in-situ formed 1 + Cu^{2+} complex

The addition of oxalate to the aqueous solution of **1** + Cu^{2+} at pH 7.4 ([**1**] = 10 μM and [Cu^{2+}] = 5 μM) showed a rapid change in color from bright pink to colorless (Fig. 3a). The characteristic absorption band of the metal complex at ~528 nm was found to be diminished in presence of oxalate. Most importantly, no interaction was observed when a wide-range of biologically relevant ionic as well as neutral analytes or antinutrients were taken as controls (Fig. 3b). Saturation in optical responses was observed upon addition of ~5 μM of analyte, which suggests high sensitivity of the present system towards oxalate (Fig. 2a). Titration studies also revealed a linear ratiometric response with oxalate, which indicates that the present system can quantify oxalate with minimum background interference (Fig. S4). Moreover, the copper chelating common anionic analytes, such as CN^-, $H_2PO_4^-$ and I^- did not show any interaction with **1** + Cu^{2+} conjugate (Fig. S5). The blank variation method suggested that the present system can detect as low as 12.5 nM of oxalate at pH 7.4 [37]. pH variation studies showed that the oxalate induced color change could be perceived over a wide range of pH (4.5–9), which is useful for real-life sample analysis (Fig. S6). Most importantly, no significant alteration in the spectral signal was observed when interaction **1** + Cu^{2+} with oxalate was checked in presence of other competing analytes (Fig. S7). On the other hand, among metal ions, only Ca^{2+}, Ni^{2+} and Zn^{2+} showed little interference in oxalate sensing (Fig. S8). This is probably due to the higher binding affinity of these metal ions (Ca^{2+}, Ni^{2+} and Zn^{2+}) towards oxalate as well as due to the poor solubility of the corresponding oxalate salts in water (Table S2). However, this does not take away the focus of the present work, as in this case, our main aim has been to determine soluble oxalate contents of various real-life samples (not in the form of insoluble metal salts). In fluorescence also, a turn-on emission response was observed selectively in presence of oxalate due to leaching of metal ions from the preformed **1** + Cu^{2+} complex (Fig. S9). Here also, Saturation in