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Highly reactive (<1 min) ratiometric "naked eye" detection of hypochlorite with real application in tap water†

Shyamaprosad Goswami,* Sima Paul and Abhishek Manna

The de-diaminomaleonitrile reaction in a simple carbazole diaminomaleonitrile hybrid (CDH) was promoted by OCl^- which disrupted the ICT mechanism by breaking the donor and acceptor linkage. This system utilized an irreversible OCl^- promoted oxidation reaction and it responded instantaneously at room temperature. The chemosensor also showed excellent performance in tap water and the "dip-stick" method.

The development of selective and sensitive signaling systems for the determination and visualization of anions with biological interest is a very attractive research topic in the chemosensing and molecular imaging fields.[1] Reactive oxygen species (ROS) are produced mainly through an electron transfer reaction from oxygen, and they play a crucial role in many physiological processes and are also implicated in many human diseases, including cancer, neurodegenerative disorders, and inflammatory processes.[2] Hypochlorite (OCl^-)/hypochlorous acid (HOCl) is one of the biologically important reactive oxygen species (ROS) and is produced in organisms by peroxidation of the chloride ions catalyzed by the heme enzyme myeloperoxidase (MPO).[3] It is implicated in a variety of pathological diseases including atherosclerosis, neuron degeneration, cystic fibrosis, arthritis, and cancers.[4,5] Hypochlorite also plays a critical role in hosting innate immunity during microbial invasion.[6] Hypochlorite is also widely used in our daily life, for example, in household bleach, disinfection of drinking water and cool-water treatment, with a concentration in the range of 10^{-5}–10^{-2} M.[7] Such highly concentrated hypochlorite solutions are a potential health hazard to humans and animals.[8] Because of the biological and environmental importance of HOCl and its anion, the development of highly sensitive and selective probes for HOCl has become an important issue.

In recent years, a number of analytical methods, such as colorimetric, luminescent, electrochemical and chromatographic methods[9] have been proposed for the detection of ClO^-. Fluorescent probes play an important role in this respect, due to their great temporal and spatial resolution capability, as well as high sensitivity, simplicity of implementation, fast response times and offering application methods for not only *in vitro* assays but also *in vivo* imaging studies.[10] Till now, only a few fluorescent probes for ClO^- have been reported.[11] Fluorescent probes are sensitive, but the signal read-out still needs the aid of bulk instruments. In comparison, colorimetric probes, in particular naked eye detection methods, have obvious advantages. Moreover, spectral studies are significantly influenced by some factors including probe concentration, sample environment and light scattering. By contrast, these factors may be potentially avoided by employing ratiometric probes, as they allow the measurement of absorbance and intensities at two wavelengths.[12] Thus, we have designed a ratiometric "naked eye" as well as fluorescent probe for the detection of ClO^-.

Generally, when a fluorophore contains an electron-donating group conjugated to an electron-withdrawing group, it undergoes intramolecular charge transfer (ICT) from the donor to the acceptor upon excitation by light. The consequence of the ICT is a red shifted emission and the compound becomes non-fluorescent. Many fluoroionophores have been designed by employing the interaction of cations with the electron-donating group.[13] After being bound to a cation, the electron-donating group loses its donating ability, so the ICT ceases, a blue shift appears and the compound becomes strongly fluorescent, which is often used for the fluorescent ratiometric determination of cations (Scheme 1).[14] Instead of the suppression of ICT through metal co-ordination with a receptor, it is also reasonable to deduce that the fluorescence intensity can be enhanced by breaking the linkage of the electron-withdrawing and electron-donating groups in the sensor molecule through a chemical reaction promoted by special analytes. However, there is no report concerning this thought, to suppress the ICT mechanism.

Bengal Engineering and Science University, Shibpur, Howrah 711103, India. E-mail: spgoswamical@yahoo.com; Fax: +91-3326682916

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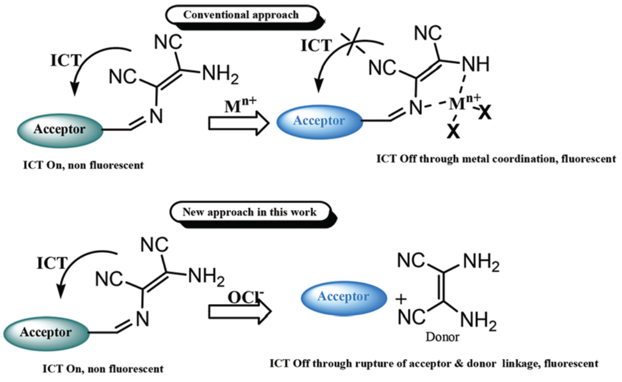


Scheme 1 Schematic approach for the detection of ClO^-.

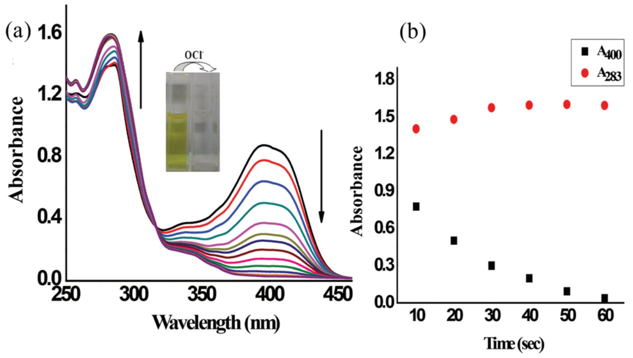
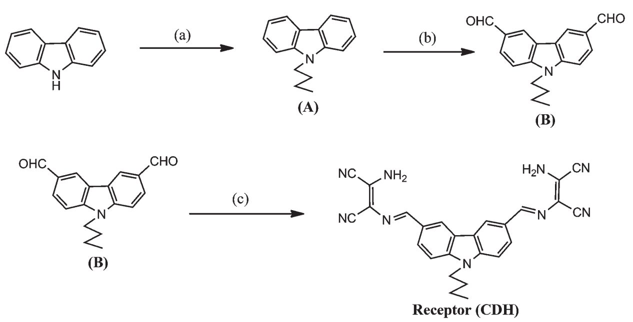


Fig. 1 (a) UV-vis absorption titration spectra of the CDH ($c = 2.0 \times 10^{-5}$ M) in the presence of OCl^- ($c = 2.0 \times 10^{-4}$ M) at pH 7.4 in $CH_3CN–H_2O$ (4 : 6, v/v). The naked eye colour change of the CDH on addition of OCl^- (inset). (b) Time-dependent absorption intensity of the CDH at 283 nm (in red) and 400 nm (in black) in the presence of OCl– (2 equiv.).

The plausible mechanism of sensing can be ascribed to the rapid de-imination of the aldehyde group by hypochlorite under mild conditions (*i.e.* ambient temperature). By utilizing the virtue of this specific deprotection reaction promoted by hypochlorite, recently W. Lin *et al.* reported a highly selective probe for hypochlorite.[15] In our receptor, we protected the aldehyde group with diaminomaleonitrile (donor), which becomes non-fluorescent due to intramolecular charge transfer. Taking advantage of the high oxidizing properties of OCl^-, our receptor, carbazole diaminomaleonitrile hybrid (CDH), behaved as a fluorescent chemosensor for OCl^-. The caged fluorescence moiety of the chemosensor was oxidatively removed by OCl^- (scheme presentation of the reaction in Fig. 4). We expected the protective group, diaminomaleonitrile, to be removed and the fluorescent carbazole aldehyde to be generated, accompanied by the "off–on" signal. If this was the case, the design approach in this work should open up a new avenue for the development of fluorescence turn-on chemodosimeters for OCl^-, by suppressing the ICT mechanism through breaking the linkage of the electron-donating and electron-withdrawing groups (Scheme 1).

The target compound was synthesized through the series of reactions detailed in Scheme 2. The intermediate compounds (A)[16] and (B)[17] were synthesized according to the reported procedures. The structure of the receptor was confirmed by ^{1}H NMR, ^{13}C NMR and ESI MS spectra (ESI†).



Scheme 2 Synthetic route of the receptor (CDH). Reagents and conditions: (a) butylbromide, K_2CO_3, KI, CH_3CN, heated to 80 °C, 4 h; (b) $POCl_3$, DMF, 100 °C, 20 h; (c) diaminomaleonitrile, ethanol, reflux, 8 h.

The sensing properties of the CDH in response to OCl^- were investigated by both UV-visible and fluorescence titration in a $CH_3CN–H_2O$ solution (4 : 6, v/v, 10 mM HEPES, pH 7.4). The CDH absorbed strongly around 283 nm and 400 nm, which was attributed to the ICT transition in CDH. However, an increasing absorption around 283 nm emerged gradually and the absorption around 400 nm decreased simultaneously with a clear isosbestic point at 316 nm, when OCl^- was added to the CDH (Fig. 1a). The bathochromic shift was apparently attributed to the ICT.[18] This phenomenon was likely due to the oxidation of the CDH and led to the formation of the carbazoledialdehyde. In fact, in the presence of 2 equiv. of OCl^-, an 82 fold enhancement in the ratiometric value of A_{283}/A_{316} (0.056 to 4.565) was achieved with respect to the hypochlorite free solution (Fig. S3b†). Under the present conditions the reaction of the CDH with OCl^- was rather fast and it reached equilibrium within 1 min after the addition of OCl^- (Fig. 1b).

In agreement with the absorption spectral change for the CDH after introducing OCl^-, the orange colored solution of the CDH became colorless on addition of only 2 equiv. of OCl^- (see the inset of Fig. 1a). Thus, this detection behavior could be easily seen with the naked eye under a normal UV lamp. The absorption spectra of the CDH probe in the presence of different anions and oxidants were investigated. As shown in Fig. S3a,† the representative species including Cu^{2+}, Co^{2+}, Hg^{2+}, Mg^{2+}, Fe^{3+}, F^-, I^-, NO_2^-, NO_3^-, SO_4^{2-}, SO_3^{2-}, PO_4^-, OH^-, H_2O_2, O^{2-}, CH_3COOOH, showed nearly no changes in the absorption spectra observed upon the addition of those species at a concentration of 40 mM, indicating that our probe showed a selective response towards OCl^- over other anions and oxidants. In the time dependent absorption spectra, it is seen that the reaction is completed within 1 min, with a rate constant of 10.4×10^{-2} s^{-1}, which strongly supports the high reactivity of the probe (Fig. S4 and S5†).

The fluorescence signaling behavior of the CDH towards OCl^- and the representative anions and oxidants was surveyed upon excitation at 316 nm. In the case of the fluorescent titration the free probe displayed two emission peaks with a

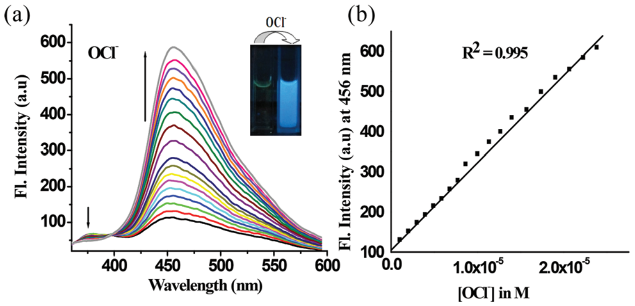


Fig. 2 (a) Fluorescence emission spectra of the CDH ($c = 2.0 \times 10^{-5}$ M) with OCl^- ($c = 2.0 \times 10^{-4}$ M) at pH 7.4 in $CH_3CN–H_2O$ (4 : 6, v/v). The naked eye fluorescence change of the CDH on addition of OCl^- (inset). (b) Plot of the fluorescence intensity *vs.* the whole range of hypochlorite concentration tested at 456 nm.

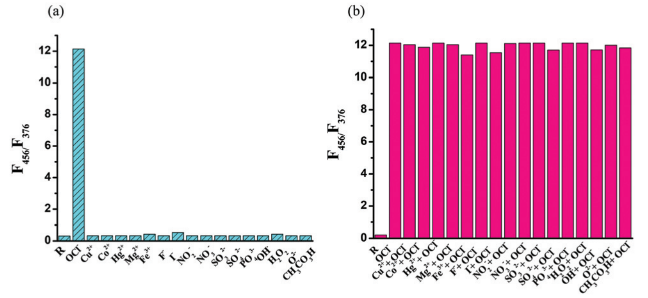


Fig. 3 (a) Comparison of the emission intensity ratio (F_{456}/F_{376}) of the CDH probe at pH 7.4 in $CH_3CN–H_2O$ (4 : 6, v/v), in the presence of various anions and oxidants. (b) Competitive fluorescent signaling of OCl^- (2 equiv.) by the CDH in the presence of various anions and oxidants (10 equiv.), $\lambda_{ex} = 316$ nm.

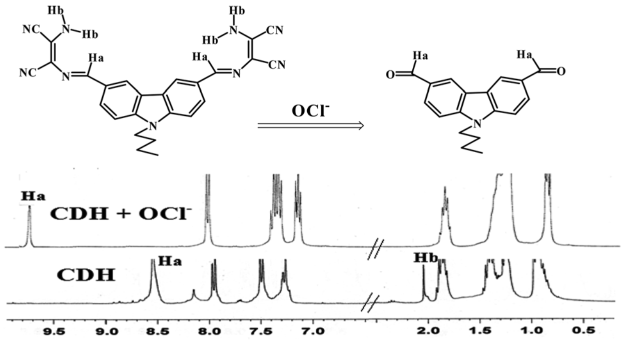


Fig. 4 Partial ^{1}H-NMR spectra of CDH and CDH + OCl^- in $CDCl_3$.

maximum intensity at around 376 nm and 456 nm ($\Phi = 0.01$), which are characteristic of the carbazole moiety. By contrast, upon addition of OCl^-, the intensity of the emission peak at 376 nm gradually decreased with the simultaneous increased intensity at 456 nm (Fig. 2a), with a clear isosbestic point at 398 nm ($\Phi = 0.65$). The fluorescence maximum was red-shifted by about 8 nm. The non-fluorescent solution exhibited blue fluorescence, when OCl^- was gradually added to the solution of the CDH (Fig. 2a, inset). A linear calibration graph of the response to OCl^- concentrations from 0.99 to 23 μM was obtained (Fig. 2b), indicating that the CDH probe can be potentially employed to quantitatively detect OCl^- concentrations. The detection limit was found to be 1.07 μM based on K × Sb1/*S*, where Sb1 is the standard deviation of blank measurements and *S* is the slope of the calibration curve (ESI†).

To test the selectivity, the CDH probe was incubated with the different anions and oxidants at pH 7.4 in $CH_3CN–H_2O$ (4 : 6, v/v). As shown in Fig. 3a, OCl^- elicited a large ratiometric signal with $F_{456}/F_{376} = 12.14$. By contrast, Fe^{3+}, I^- and H_2O_2 induced a very modest ratiometric response with $F_{456}/F_{376} < 0.45$, and the remaining species caused only negligible changes in the emission ratio ($F_{456}/F_{376} < 0.3$). Thus, these data indicated that the probe has high selectivity for OCl^-. Practical applicability of the CDH as a OCl^- signaling device was verified by competitive signaling experiments with different anions and oxidants (Fig. 3b). The CDH probe exhibited unaltered signaling for OCl^- in the presence of commonly encountered anions and oxidants (except Fe^{3+}, I^- and H_2O_2, which interfere slightly).

The ratiometric responses of the CDH probe on addition of OCl^- at different pH values were also investigated. Fig. S1† depicts the effect of the pH, which reveals that the detection of hypochlorite by the CDH is pH-dependent.

To confirm the ratiometric response of the CDH towards OCl^-, the reaction of the CDH with 2 equiv. OCl^- was carried out in $CH_3CN–H_2O$ (4 : 6, v/v). After stirring at ambient temperature for 4 h, the major product was purified by chromatography on a silica gel column and was subjected to ^{1}H NMR, ^{13}C NMR and ESI TOF mass analysis (ESI†). The partial ^{1}H NMR spectra of the CDH and the resultant compound of CDH and OCl^- are shown in Fig. 4. The resonance signal at δ = 2.050 ppm in the CDH was attributed to the primary amine protons. Upon addition of 2 equiv. of OCl^- a new signal at δ = 9.810 ppm appeared corresponding to the aldehyde protons and the resonance signal at δ = 2.050 ppm completely disappeared. Meanwhile, carbazole dialdehyde was generated due to the de-diaminomaleonitrile reaction in CDH which occurred in presence of OCl^-. In the ^{13}C NMR spectra of CDH and CDH + OCl^-, we found that the four peaks at 110.01, 114.70, 115.25 and 125.07, corresponding to the diaminomaleonitrilic carbon, disappeared and the peak at 164.35 was shifted to 179.35 (generation of aldehydic carbon). In addition, the mass spectrometry analysis after addition of OCl^- showed that the peak at *m*/*z* 459.47 disappeared and a new peak appeared at *m*/*z* 279.32 corresponding to carbazole dialdehyde.

Many sensors for OCl^- detection can only be performed in solution, which limits their application under special circumstances, such as on-site detection *in situ*. To demonstrate the practical application of our sensor, we prepared TLC plates of the CDH to determine the suitability of a "dip-stick" method for the detection of OCl^- (Fig. 5(i)).

Hypochlorite is used in many industrial processes and also in daily life. Therefore, the detection of hypochlorite in water is of interest. We analyzed OCl^- in tap water and distilled water. The experimental results showed that this sensing

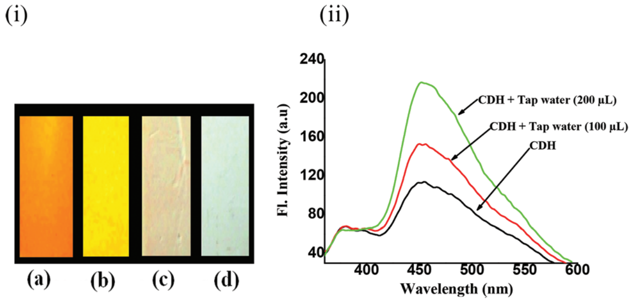


Fig. 5 (i) Photograph of the TLC plates of the CDH in various concentrations of OCl^- ($\times 10^{-3}$ M) (a) 0, (b) 5, (c) 9, (d) 11. (ii) Competition fluorescence emission spectra of CDH ($c = 2.0 \times 10^{-5}$ M) itself, in the presence of 100 μL, 200 μL tap water at pH 7.4 in $\text{CH}_3\text{CN–H}_2\text{O}$ (4 : 6, v/v).

system gave no fluorescence response to the deionized water (Fig. S7†). Thus, deionized water samples had no interference in the performance of this sensing system. Nevertheless, the addition of tap water (100 μL) led to a significant increase in the fluorescence intensity of the sensing system, and a bigger change was observed when increasing the amount of tap water (Fig. 5(ii)), which further revealed that such a sensing system was sensitive towards hypochlorite, even in real water samples. The concentration of hypochlorite in tap water was determined to be 0.256 mol L^{-1} from comparing the fluorescence intensity of the CDH in the presence of tap water with the titration data.

In conclusion, a new carbazole based "naked eye" and ratiometric fluorescence probe, CDH, was developed for selective and rapid detection of OCl^- at physiological pH of 7.4 in aqueous media. The chemical reaction was fast at room temperature and irreversible. In this reaction, the addition of OCl^- to the CDH disrupted the ICT mechanism by opening a new avenue for the development of a fluorescence turn-on chemodosimeter for OCl^-. This chemodosimeter is also a good candidate with great potential for the selective detection of OCl^- in the presence of different common ions and oxidants. It also showed good performance in tap water samples.

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