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Communication

A Selective Colorimetric and Fluorescent Probe for Detection of ClO^- and its application in bioimaging

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Disperse Violet 26 commercially available fluorochrome as an on-off fluorescent probe for the detection of ClO^-, which is one of the biologically important reactive oxygen species (ROS), in HEPES: CH_3CN = 1:1 (V/V pH=7.0) with an excellent selectivity and sensitivity for ClO^- over other analytes.

Hypochlorous acid (HOCl) is one of the biologically important reactive oxygen species (ROS).[1-4] Due to it's subacidity, HOCl usually take a spontaneous hydrolysis reaction in the neutral solutions of pH 7.0 resulting in the formation of free ClO^-. Biologically, the ClO^- ion is believed to be produced by hydrogen peroxide and chloride ions in activated neutrophils catalyzed by a heme-containing enzyme myeloperoxidase (MPO).[5,6] Hypochlorite plays an essential role in the immune system due to significant antibacterial properties.[7] However, there is growing evidence that excessive of hypochlorite can lead to tissue damage and diseases, such as neuron degeneration [8], cancer [9,10], cardiovascular diseases [11], and arthritis [12]. This may be ascribed to the fact that hypochlorite in the physiological condition can react with DNA, RNA, fatty acids, cholesterol, and proteins.[7] As a decolorizer and disinfector, hypochlorite is also widely used in our daily life.[13] Therefore, it is of vital practical significance to detect hypochlorite through highly sensitive and selective methods. There have been some successful examples reported very recently such as colorimetric, luminescent, electrochemical and chromatographic methods.[14-26] Even so, fluorescent probe detection is a promising method for detection of hypochlorous acid because of the low cytotoxicity of fluorescent probe which can realize detection in living cells.[27-29] For example, Wu et al have reported a BODIPY-based fluorescent probe (detection limit was 17.7 nM) which be successfully applied to fluorescence image in RAW264.7 cells.[30] Another fluorescein-based probe for hypochlorite anion (detection limit was 40 nM) was synthesized by yin et al, and it was used for labeling in organisms.[31] All above probes were cost-consuming and time-consuming due to the certain organic synthesis processes.

In this work, a commercially available organic pigment, Disperse Violet 26 (abbreviated as DV26 afterwards, Scheme 1) was developed as a high selective fluorescence probe for hypochlorite ion over other anions. This probe work well at physiological pH and has a high selectivity and sensitivity for ClO^- over other analytes. These desirable attributes render the sensor suitable for detection of ClO^-.

The recognition ability of DV26 was investigated by the UV–Vis and fluorescence spectra. To verify the selectivity of probe to hypochlorite, the absorbance study in aqueous solution were carried out. The absorbance change that DV26 undergoes upon the addition of various analytes were shown in Fig. 1. After adding 1000 μM of analytes, such as H_2O_2, ClO_2^-, $ONOO^-$, F^-, ClO_3^-, CN^-, NO_2^-, S^{2-}, SCN^-, MnO_4^-, ClO_4^-, CO_3^{2-} and $P_2O_7^{4-}$ to the HEPES:CH_3CN = 1:1 (V/V pH=7.0) solution containing probe (45 μM) for 5 min, only ClO^- induced a decrease of absorption peak at 544 nm and 586 nm. Accordingly, the addition of ClO^- to probe produces a colorimetric change from purple to colorless, which can be detected by naked-eye. Other analytes show neglectable change in absorbance spectra under the same conditions.

We also carried out titration experiment for ClO^-. With increasing ClO^- concentration (0–110 μM), a gradual decrease of the absorption peak at 544 nm and 586 nm and a progressive increase of a new absorption band at around 300 nm by addition of ClO^- were observed (Fig. 2). All these indicate the formation of a new species.

The fluorescence response of DV26 for ClO^- was also examined. After adding 500 μM of analytes, such as H_2O_2, ClO_2^-, $ONOO^-$, F^-, ClO_3^-, CN^-, NO_2^-, S^{2-}, SCN^-, MnO_4^-, ClO_4^-, CO_3^{2-} and $P_2O_7^{4-}$ to the HEPES: CH_3CN = 1:1 (V/V pH=7.0) solution containing probe for 5 min, only ClO^- induced a remarkable fluorescence decrease at 625 nm, which also results in a visual fluorescence change (from pink to colorless) under illumination with a UV 365 nm lamp. Other analytes show neglectable change in fluorescence spectra under the same conditions. (Fig. 3, Fig. S1, ESI†)

More, we carried out a detailed investigation on the DV26 recognition of ClO^- on fluorescence Spectrometer. Fig. 4 shows a regular change in the fluorescence spectrum when the ClO^- solution was added to the CH_3CN-HEPES buffer (pH=7, v:v=1:1) containing the probe (35 μM). The probe is strong-fluorescent in the absence of ClO^-, however, an increase in the ClO^- concentration caused a dramatic change in the fluorescence spectra. A significant decrease (23-fold) of the fluorescence intensity at 625 nm was observed.

Time-dependent modulations in the fluorescence spectra of probe were monitored in the presence of 10 equiv. of ClO^- (Fig. 5). The kinetic study showed that the reaction was completed within 4 min for ClO^-, indicating that probe reacts rapidly with ClO^- under the experimental conditions. This unprecedented fast response could provide the possibility of quantitative detection without any pretreatment of samples.

The pH range for the determination of ClO^- was also studied. Figure S2 ESI† showed the fluorescence intensity obtained for the free probe and probe-ClO^- in different pH values. It was

obvious that the fluorescent signal of the probe in the CH_3CN-HEPES buffer (pH =7.0, v:v=1:1) in the pH range 2~13. When the solution pH is in the range of 3-5 or 9-11, ClO^- induced a fluorescence intensity for probe such that there was no quenching or only partial quenching. Therefore, the pH range of 6–8 is effective for this probe and neutral pH was used for further studies.

To investigate the detection limit of the probe for ClO^-, probe (35 μM) was treated with various concentrations of ClO^- (0–80 μM) and the fluorescence intensity at 625 nm was plotted as a function of ClO^- concentration (Fig. S3, ESI†). The fluorescence intensity of probe is linearly proportional to the ClO^- concentrations, and the detection limit is 0.037μM, based on the definition by IUPAC 139 (C_{DL}= 3 Sb/m).[32] The detection limit indicates that commercially available fluorescence probe Dv26 show a certain sensitivity towards ClO^- that is comparable to the other synthetic probes for ClO^- (Fig. S4, ESI†).[33-38]

The reaction mechanism of the present system was studied. We presumed that the color change and fluorescence quenching could be attributed to the oxidation of DV26 to its azo derivative. As we all known, aromatic amines may be oxidized to the corresponding azo compound in the presence of an oxidant. We speculated the mechanism is based on a specific reaction promoted by hypochlorite: namely, hypochlorite is a strong oxidant, it can oxidize amino of DV26 to form azo product. However, C-N bond in the azo product is easily broken to form a radical, which could be combined with a chlorine radical to form compound 2 (Scheme 2). To elucidate the detailed signal mechanism, ESI-MS analysis of isolated product from complete reaction mixture of probe with ClO^- was carried out. The identification of stable product in the ESI-MS analysis made it possible to propose the signaling mechanism: a peak at m/z = 541.08, corresponding to [compound 2 + Na]$^+$, is clearly observed (Fig. S5, ESI†). The reaction is different from other recognition mechanisms, such as oxidation reactions of *p*-methoxyphenol to benzoquinone,[33,34] utilizing the oxidative deoximation reaction of luminescent oxim[35,36] oxidation reactions of benzidinediimine to dibenzoyl,[37] the oxidized ring opening reaction[38] et al. This made it become possible that organic complexes containing amino can be used to design hypochlorite fluorescence probes.

The ability of probe to detect ClO^- within living cells was also evaluated by laser confocal fluorescence imaging using a Leica TCS SP5 laser scanning microscope. The optical window at the yellow channel (600–700 nm) was chosen as a signal output. As shown in Fig. 6a, under selective excitation at 488 nm, HepG2 cells incubated with 20 μM probe for 30 min at 37 ℃ showed pink fluorescence. In a further experiment it was found that HepG2 cells displayed no fluorescence when the cells were first incubated with 20 μM of probe for 30 min at 37 ℃ and then incubated with 40 μM NaClO (Fig. 6c). These cell experiments show the good cell-membrane permeability of probe, and it can thus be used to mark ClO^- within living cells.

In summary, we have developed a colorimetric and fluorescent probe for the detection of ClO^- "fluorescence turn-off" over other analytes in aqueous solution. This probe is based on a commercially available and cheap luminescent dye DV26 which has a high selectivity and sensitivity for ClO^- over other analytes in HEPES: CH_3CN = 1:1 (V/V pH=7.0). The probe displayed a dramatic change in fluorescence intensity and absorbance intensity when ClO^- is added to the system. Furthermore, the system is used to bioimaging. This work will therefore prove useful for developing organic dye or fluorescent dye as chemosensors.

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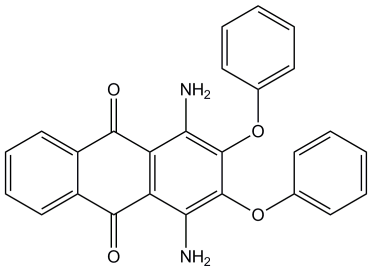
Inserting Graphics



Scheme 1 The structure of the DV26.

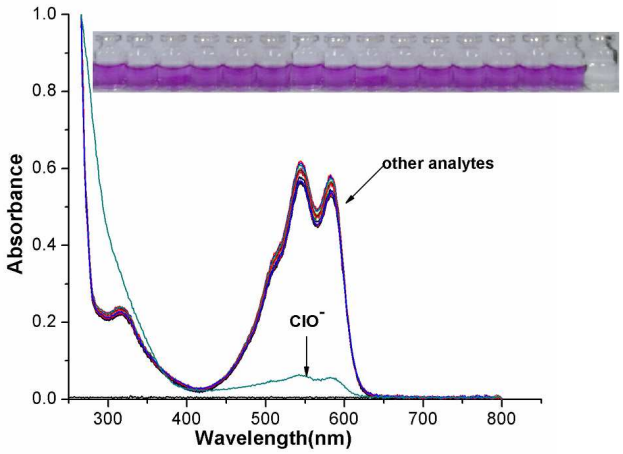


Fig. 1 UV/vis absorption spectra of probe (45 μM) in HEPES: CH_3CN = 1:1 (V/V pH=7.0) in the presence of 1000 μM analytes (H_2O_2, ClO_2^-, $ONOO^-$, F^-, ClO_3^-, CN^-, NO_2^-, S^{2-}, SCN^-, MnO_4^-, ClO_4^-, CO_3^{2-} and $P_2O_7^{4-}$). Inset: a color change photograph for ClO^- and other analytes.

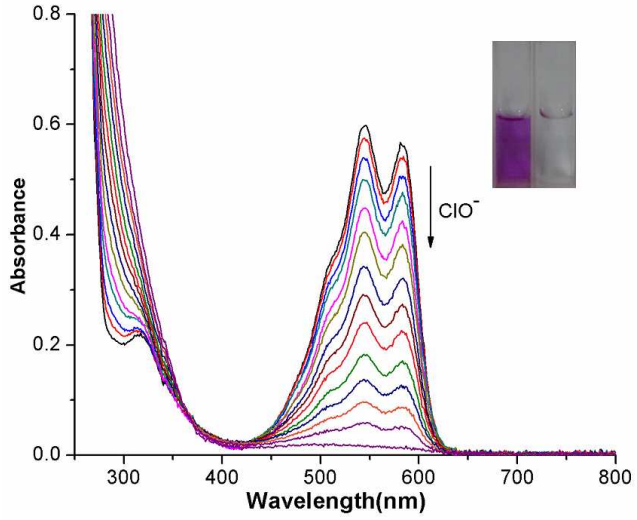


Fig. 2 The absorption spectra of the probe (45 μM) in the presence of various concentrations of ClO^- (0–110 μM) in HEPES: CH_3CN = 1:1 (V/V pH=7.0)

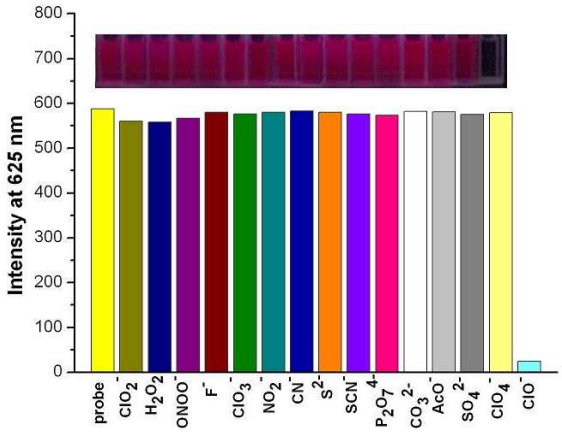


Fig. 3 Optical density graph of the probe (35 μM) at 625nm upon the addition of several analytes (500 μM) for 5 min in HEPES: CH_3CN = 1:1 (V/V pH=7.0) (λ_{ex}=520 nm, slit: 5/5 nm). Inset: a color change photograph for ClO^- and other analytes (H_2O_2, ClO_2^-, $ONOO^-$, F^-, ClO_3^-, CN^-, NO_2^-, S^{2-}, SCN^-, MnO_4^-, ClO_4^-, CO_3^{2-} and $P_2O_7^{4-}$).

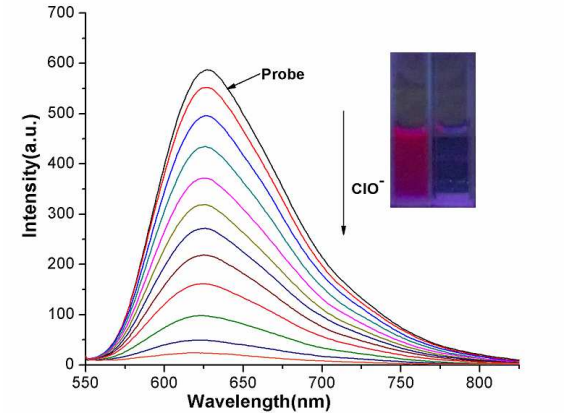


Fig. 4 Fluorescence spectra of probe (35 μM) in the presence of various concentrations of ClO^- (0–80 μM) in HEPES: CH_3CN = 1:1 (V/V pH=7.0) (λ_{ex} = 520 nm, slit: 5 nm/5 nm).

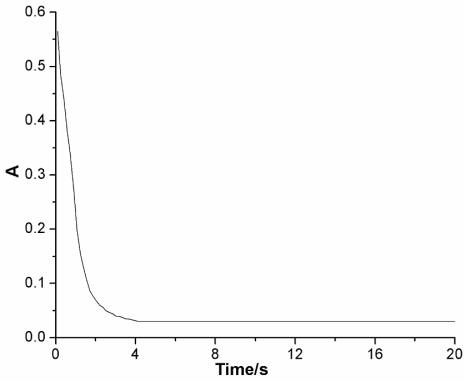
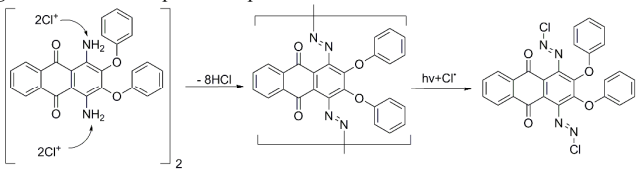


Fig. 5 Reaction time profile of probe and ClO^-.



Scheme 2. The mechanism of chemosensor.

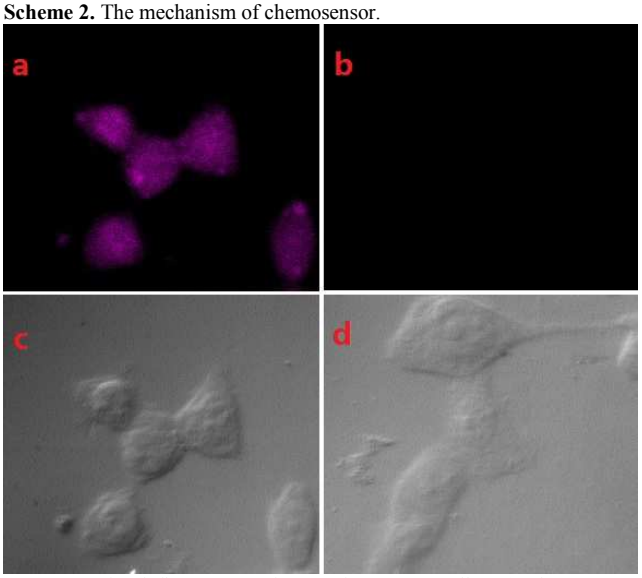


Fig. 6 Confocal fluorescence images in HepG2 cells. (a) Fluorescence image of HepG2 cells with adding DV26 (20 μM) and its bright field image (c); (b) Fluorescence image of HepG2 cells incubated with 20 μM DV26 for 30 min at 37 °C, then incubated with 40 μM ClO^- for 30 min at 37 °C and its bright field image (d)..

Notes and references

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