Azo dye-based latent colorimetric chemodosimeter for the selective detection of cyanides in aqueous buffer

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ARTICLE INFO

ABSTRACT

We report a novel latent colorimetric probe (**1**) for cyanide, which possesses a masked phenol in the para position of an azo dye group. Upon the addition of cyanides, probe **1** undergoes a ring opening reaction through the Michael addition of cyanide ion and a subsequent [1,3]-sigmatropic rearrangement reaction to give rise to a stable free phenol, whose signal is transduced to the azo dye unit producing a dramatic color change.

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1. Introduction

Industrially released cyanide ions are widely distributed in the environment and do harm to organisms by absorption through the lungs, gastrointestinal tract, and skin, leading to vomiting, convulsion, loss of consciousness, and eventual death. Therefore, highly selective probes for cyanide anions are of considerable interest due to their applicability to environmental cyanide detection [1] and to the pathological bioimaging of the anions [2]. In spite of much effort in the development of efficient probes for cyanides, it is not easy to develop cyanide probes that are operable in water. Because the charged cyanide anions are highly solvated in water, the thermodynamic factors, particularly enthalpy, are not favorable for the cyanide-probe complex formation in reversible chemosensors [3]. To overcome the enthalpic cost for the cyanide-probe complexation in aqueous solution, a novel chemodosimeteric approach was introduced, which utilizes the innate nucleophilicity [4] or the strong ligation ability [5] of cyanide anions. The strong nucleophilicity of cyanide would make the cyanide-probe complex more feasible and often work as an irreversible probe, a reaction-based chemodosimeter [6].

We have developed conjugated enone systems for the detection of cyanides in organic solvent [7]. Cyanide readily reacted with conjugated enone through the Michael reaction and changed the hybridization environment around a probe to induce the characteristic luminescence or chromophore properties of the designed probes. However, the fluorescence or color changes of the probes in aqueous solvent were not eminent due to the initial luminescence or color of probe itself. Herein, we report an azo dye-based latent colorimetric probe (**1**) possessing a masked phenol in the para position of an azo dye group, which exhibited a dramatic color change for cyanide in aqueous solution.

2. Experimental

2.1. General

All reagents and solvent were purchased from commercial source and used without further purification, if not otherwise stated. All reactions were carried out on the magnetic stirrers and their reaction process was monitored on thin layer chromatography (TLC). Purification was performed by flash chromatography with silica gel 60 (230–400 mesh). Absorption spectra were taken on an Agilent 8453 spectrophotometer. NMR measurements were conducted in 200 or 300 MHz (^{1}H) and a 50 or 75 MHz (^{13}C) spectrometer. The solvent for the NMR measurements was dimethyl sulfoxide (DMSO-d_6) or methanol (CD$_3$OD). All peaks were given as δ in ppm and were measured relative to the signals of residual nondeuterated solvent peak. The following abbreviations were used to explain the multiplicity: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet.

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2.2. Preparation of (E)-2-hydroxy-5-(4-nitrophenyldiazo)benzaldehyde

The azosalicylaldehyde were prepared according to the modified literature procedure [8] from 4-nitroaniline (6.90 g, 50 mmol), sodium nitrite (4.14 g, 60 mmol) and HCl (34 mL, 400 mmol) in water (150 mL). This mixture was stirred for 0.5 h in an ice bath. Then, a solution of sodium hydroxide (8 g, 200 mmol) and salicylaldehyde (6.73 g, 60 mmol) in water/methanol (55 mL/30 mL) was slowly added to the above solution, which was stirred for 1 h at 0 °C. The precipitate was collected, which was recrystallized in ethanol to afford desired product as the orange powder in 30% yield.

^{1}H NMR (200 MHz, DMSO-d_6): δ 11.77 (s, 1H), 10.39 (s, 1H), 8.43 (d, 2H, J = 9.0 Hz), 8.27 (d, 1H, J = 2.4 Hz), 8.17 (dd, 1H, J = 8.8 Hz, 2.4 Hz), 8.06 (d, 2H, J = 9.0 Hz), 7.24 (d, 1H, J = 8.8 Hz).

2.3. Preparation of 1

(E)-2-Hydroxy-5-(4-nitrophenyldiazo)benzaldehyde (0.54 g, 2.0 mmol) and metylvinyl keton (MVK) (0.34 mL, 4.0 mmol) were dissolved in 10 mL of THF and then DMAP (0.25 g, 2.0 mmol) were added at rt. The reaction mixture was reflux for 48 h to afford orange precipitates. Resulting precipitates were filtered out and purification of the mixture by column chromatography on silica gel (DCM as an eluent) gave the desired product in 71% yield.

^{1}H NMR (200 MHz, DMSO-d_6): δ 8.44 (d, 2H, J = 8.0 Hz), 8.10 (d, 2H, J = 8.0 Hz), 8.03–7.98 (m, 2H) 7.87 (s, 1H), 7.11 (d, 1H, J = 8.0 Hz) 5.08 (d, 2H, J = 1.0 Hz) 2.42 (s, 3H).

^{13}C NMR (50 MHz, CDCl$_3$): δ 195.7, 159.1, 155.7, 148.5, 147.3, 132.8, 131.4, 128.5, 124.8, 124.0, 123.3, 120.7, 117.1, 65.1, 25.1.

HRMS (FAB$^+$, m-NBA): m/z obsd 324.0982 ([M+H]$^+$, calcd 324.0984 for C$_{17}$H$_{14}$N$_3$O$_4$).

2.4. Preparation of 1-CN

To the solution of 1 (0.10 mmol) in DCM/MeOH (10:1, 1.0 mL) was added Bu$_4$NCN (0.15 mmol) and the reaction mixture was stirred at rt for 30 min. Evaporation of all the volatiles and purification of the product by short column chromatography on silica gel (DCM/MeOH, 20:1, v/v, R_f = 0.3) gave the desired product as a red solid in 20% yield.

^{1}H NMR (200 MHz, CD$_3$OD): δ 8.42 (d, 2H, J = 9.2 Hz), 8.04 (d, 2H, J = 9.2 Hz), 7.94 (dd, 1H, J = 8.6 Hz, J = 2.2 Hz), 7.89 (d, 1H, J = 2.2 Hz), 7.07 (d, 1H, J = 8.6 Hz), 2.00 (s, 3H), 1.63 (s, 3H).

HRMS (MALDI$^+$, DHB): m/z obsd 373.0904 ([1-CN+Na]$^+$, calcd 373.0907 for C$_{18}$H$_{14}$N$_4$O$_4$Na).

2.5. General UV–vis measurement

Stock solution (10 mM) of 1 in DMSO was prepared and used for UV–vis measurement by dilution with 10% HEPES/DMSO buffer (0.10 M, pH 7.4). For the UV–vis measurement, sample solutions were obtained by mixing an appropriate amount of stock solution of 1 (10 mM in DMSO) with a specified amount of each anion and finally diluted with 10% HEPES/DMSO buffer (0.10 M, pH 7.4). Usually, UV–vis experiment was conducted under the concentrations of 1 (10 μM) and anion (20 mM) in 10% HEPES/DMSO buffer (0.10 M, pH 7.4).

2.6. Binding stoichiometry

The binding stoichiometry of 1 with cyanide ions was determined by a Job's plot. For the Job's plot analyses, a series of solutions with varying mole fraction of metal ions were prepared by keeping the total concentration of 1 and cyanide constant (10 μM). UV–vis absorbance spectra of the solutions were measured for each sample
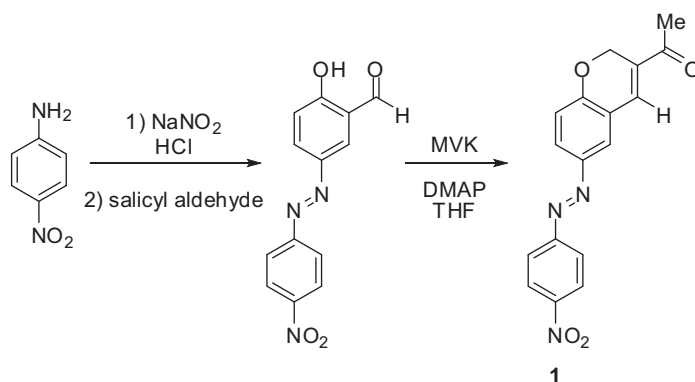
Scheme 1. Synthesis of 1.

from 300 to 700 nm. The absorbance at A_{max} 560 nm was monitored for each spectrum and the values of $A_{560 nm}$ were plotted against X_1 (mole fraction of 1).

3. Results and discussion

Latent probe 1 was prepared from p-nitroaniline via 3 steps (Scheme 1). Azotization of salicylaldehye [8] and subsequent Baylis–Hillman condensation with methylvinyl ketone (MVK) afforded the desired probe (1) in 21% overall yield.

Fig. 1 shows the time-dependent UV–vis changes of 1 (10 μM) upon the addition of cyanides (20 mM) with a prominent bathochromic shift (ΔA 170 nm) in 10% HEPES/DMSO solution (0.10 M, pH 7.4). In the presence of cyanide ions, the absorbance band of 1 at 390 nm was shown to decrease while a new band increased at 560 nm with a clear isosbestic point at 445 nm. The ratiometric UV–vis response induced a dramatic color change of colorless solution of 1 into the dark violet solution of 1-CN complex. Probe 1 readily reacted with cyanides and its reaction was complete within 5 h with the second-order rate constant of k_2 = 6.5 × 10^{-3} M^{-1} s^{-1} at 25 °C (Fig. S7, Supplementary data).

In order to assess the anion selectivity of probe 1, we screened a variety of anions including thiol containing amino acids. Probe 1 exihibited a selective ratiometric response to cyanide (A_{560}/A_{390} = 10), whereas other anions and cysteine did not induce any detectable UV–vis changes (A_{560}/A_{390} < 0.06) (Fig. 2). The relative absorbance ratio changes were more than 167, which is one of the largest values ever reported with a significant bathochromic shift (ΔA 170 nm). To confirm the selectivity of probe 1 for cyanide, competitive assays were performed by adding CN$^-$ (20 mM) to
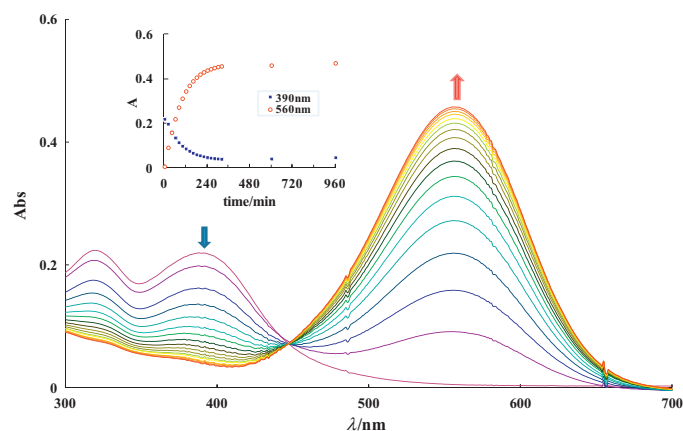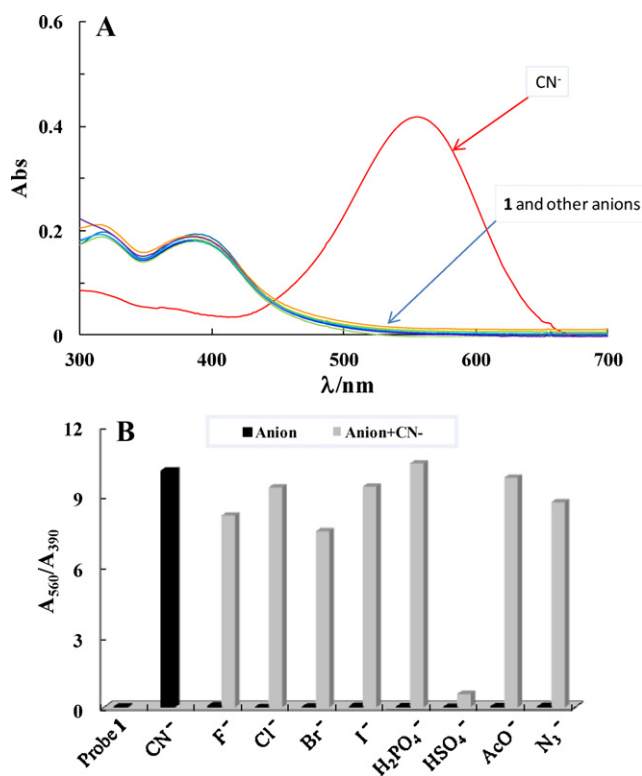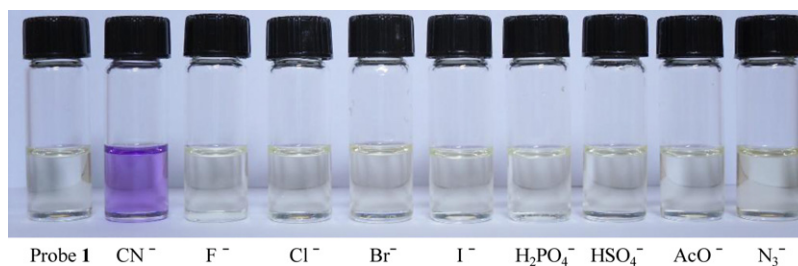

Fig. 1. Time-dependent UV–vis spectra of 1 (10 μM) upon the addition of cyanide (20 mM) in 10% HEPES/DMSO buffer (0.10 M, pH 7.4). Inset: its kinetics.
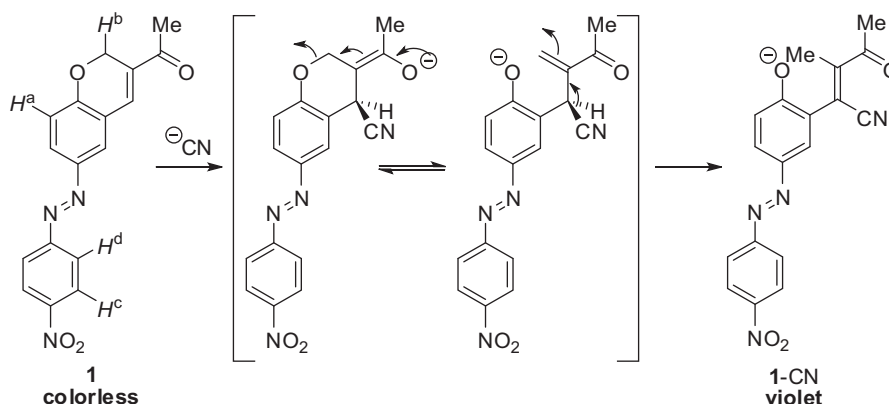
Fig. 2. (A) UV–vis spectra of **1** (10 μM) with various anions (20 mM) in 10% HEPES/DMSO buffer (0.10 M, pH 7.4) and (B) ratiometric responses of **1** in the competitive binding assays.
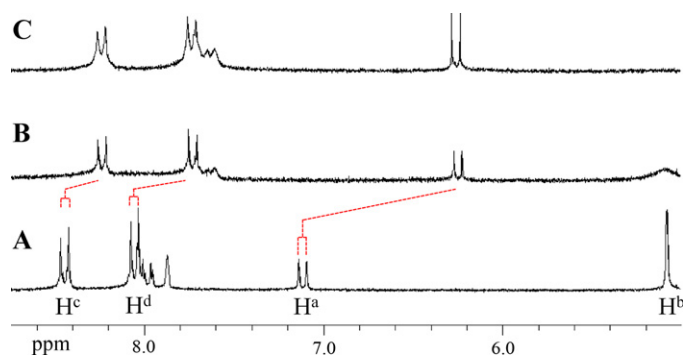
the mixtures of **1** (10 μM) and other anions (20 mM) in 10% HEPES/DMSO buffer (0.10 M, pH 7.4). The addition of CN⁻ to the mixtures of **1** and other anions induced as significant responses as that of **1**-CN solution (Fig. 2B). These competitive assays have

Fig. 4. Partial ^{1}H NMR spectra of **1** (10 mM) in the absence (A) and presence of 1.0 equiv (B), and 2.0 equiv (C) of cyanides in DMSO-d_6/D$_2$O (50:1, v/v).

also shown a consistent result for the selectivity of probe **1** toward cyanide except for HSO$_4^-$. The acidity of hydrogen bisulfate is thought to interrupt the nucleophilicity of cyanide.

The significant UV–vis changes of **1** could be applied for the detection of cyanide by the naked eye (Fig. 3). Upon the addition of cyanide anions to **1** (10 μM) in 10% aqueous DMSO, the chemodosimeter (**1**) turned its color from colorless into dark violet. In contrast, other anions did not induce any significant chromophoric changes.

To elucidate the reaction mechanism, ^{1}H NMR spectra of **1** were investigated in the absence and presence of cyanides. Upon the addition of 1.0 equiv of CN⁻ to **1**, a new set of spectra was observed, which was shown to be stable in excess CN⁻ (Fig. 4). Both the characteristic upfield shift of aromatic proton (H^a) of **1** (7.11–6.25 ppm) and the disappearance of allylic proton (H^b at 5.08 ppm) indicated that the Michael addition of CN⁻ to **1** followed by ring opening reaction took place to afford a free phenol group as suggested in a similar system [9]. To our surprise, however, we could not find a methine proton at the benzylic position of the intermediate but observed

Fig. 3. Nakey eye detecion of cyanide ions by **1** (10 μM) in 10% HEPES/DMSO buffer (0.10 M, pH 7.4).

Scheme 2. A proposed response mechanism of **1** to cyanide.

two different methyl protons around 2 ppm [10]. This indicates that the intermediate underwent a subsequent [1,3]-sigmatropic rearrangement reaction and gave rise to the more stable enone (**1**-CN) by conjugation with the aromatic group (Scheme 2). The free hydroxyl group as observed by the significant upfield shifts of the azonitrophenyl protons (H^c and H^d) should cause a significant dipole moment change in the azo dye and induce a dramatic color change in the chemodosimeter. The stoichiometry between **1** and CN$^-$ was determined by the Job's plot [11], which showed one to one binding between **1** and CN$^-$ (Fig. S8, Supplementary data). The mass spectra showed corroborative evidence for the formation of **1**-CN conjugate at m/z obsd 373.0904 ([**1**-CN+Na]$^+$, calcd 373.0907 for $C_{18}H_{14}N_4O_4Na$) (Fig. S6, Supplementary data).

According to the experimental evidence, we propose the reaction mechanism of **1** with CN$^-$ (Scheme 2). The nucleophilic addition of CN$^-$ to **1** through the Michael reaction induced a ring opening reaction via enol-keto tautomerization and a subsequent [1,3]-sigmatropic rearrangement reaction gave rise to the final free hydroxy azo compound, which displayed a dramatic violet color of **1**-CN conjugate in aqueous buffer.

The limit of detection (LOD) of CN$^-$ was determined to be 1.27 mM at $3\sigma/m$, where σ is the standard deviation of blank measurement and m is the slope obtained from the linear plot of **1** against CN$^-$ (Fig. S9, Supplementary data) [12]. To investigate the effect of pH on the ratiometric response of **1**, the ratiometric UV–vis changes of **1** and **1**-CN were measured at various pHs. In the absence of CN$^-$, the colorless probe (**1**) showed no detectable UV–vis changes in a wide range of pHs (pH 3–11). In the presence of CN$^-$, however, dramatic ratiometric UV–vis changes were observed with concomitant color changes into violet at pH 4–11 (Fig. S10, Supplementary data).

4. Conclusion

We prepared a latent colorimetric probe (**1**) for cyanide. Upon the addition of cyanides, probe **1** underwent a ring opening reaction through the Michael addition of cyanide and a subsequent [1,3]-sigmatropic rearrangement reaction gave rise to a stable free phenol group, whose signal was transduced into the azo dye unit to afford dramatic color changes. The chemodosimeter exhibited a highly selective and colorimetric response to cyanide ions over other anions. The dramatic color changes of **1** allowed us to detect toxic cyanide anions even by the naked eye in aqueous solution.

Acknowledgements

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.snb.2012.08.038.

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[10] Instead of vinyl protons, two methyl protons at 2.0 and 1.6 ppm are observed, which indicates that a subsequent sigmatropic rearrangement reaction took place (Fig. S3, Supplementary Data). Further research on the different behaviour of methylvinyl ketone and cyclopentenone systems will be reported in due course.

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