Visual Detection of Cyanide through Intramolecular Hydrogen Bond

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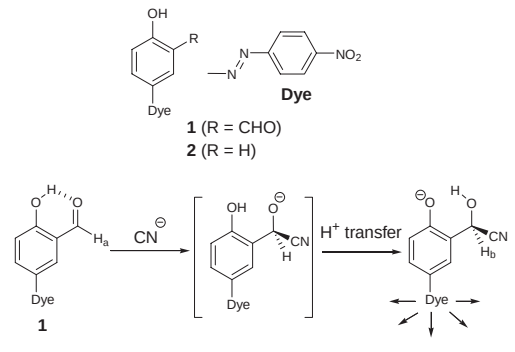
An azo compound based on salicylaldehyde displays a red color upon binding to the cyanide anion with micromolar sensitivity. The high affinity is caused by an intramolecular hydrogen bond of phenol hydrogen to carbonyl oxygen, which stabilizes the anionic character of the intermediate during the nucleophilic attack of a cyanide anion.

Pyridoxine or vitamin B_6 is an important dietary requirement. Its aldehyde form of the pyridoxal phosphate is implicated in many enzyme-catalyzed reactions of amino acids and amines as an important coenzyme.[1] The key step for the reactions is the carbonyl activation by an ideally located phenol proton to provide a general acid catalysis during the nucleophilic reactions. This kind of intramolecular hydrogen-bonding activation is commonly observed in the *o*-hydroxy-substituted benzaldehyde (salicylaldehyde)[2a] or benzimine.[2b]

There is a growing interest in sensing the toxic cyanide anion by coordination[3] or covalent bonds.[4] The process of cellular respiration in mammals is inhibited by the cyanide anion, which is known to interact strongly with a heme unit in the active site of cytochrome a_3.[5] Therefore, it is very important to sense the lethal cyanide in a fast assay method. Herein, we report a facile visual detection method for the cyanide with a salicylaldehyde-based azo chromophore **1**.

To utilize an intramolecular hydrogen bond of the phenol proton for cyanide sensing, we introduce a salicylaldehyde as an electrophile for the anion and an azo dye functional group as a chromophore. An azo dye host **1**, possessing a salicylaldehyde unit, was synthesized according to the literature procedure.[6a] Compound **2**, without an aldehyde functionality, was also prepared as a reference molecule (Scheme 1).[6b] It is expected that cyanide is detectable by nucleophilic attack toward a carbonyl function, which is activated by the phenol proton of general acid, then followed by fast proton transfer of phenol hydrogen to the developing alkoxide anion of **1**.



Scheme 1. Hosts and a plausible sensing mechanism of cyanide.

As a preliminary study, we have investigated the ^{1}H NMR spectra of host **1** in the presence of the nucleophile, cyanide anion. It was found that the aldehyde proton (initially around δ 10.4) was dramatically shifted toward δ 5.6 upon addition of cyanide at room temperature, this chemical shift being consistent with a cyanohydrin form due to the nucleophilic attack of cyanide anion toward the carbonyl group of **1** (Figure 1).

In fact, the phenol proton of **1** plays a dual role for the incoming anions as a general acid[4b] to assist a nucleophilic attack of the anion or as an acid itself for the basic anion. It is plausible that the deprotonation of the phenol proton occurs in both cases and creates a color change in the azo chromophore. In the present case, it is observed that cyanide is mainly operating as a nucleophile.[7]

These cyanide anion sensing phenomena were analyzed by UV–vis titration in dimethyl sulfoxide. The light yellow color ($\lambda_{max} = 380$ nm, $\varepsilon_{380nm} = 2.01 \times 10^4\,M^{-1}\,cm^{-1}$) of **1** was changed to the dark red color ($\lambda_{max} = 540$ nm) upon addition of cyanide anion. (Figure S1) This bathochromic shift is due to the conversion of phenol to phenolate anion in an azo dye molecular sensor.[8] A plot of A/A_o vs [G]/[H] has shown that the host–guest complexation reaches a saturation point around 2 equiv. of cyanide, yielding a micromolar sensitivity of $K_d = 1.95\ (\pm 0.56) \times 10^{-6}$ M. To the best of our knowledge, this value is one of the highest binding affinities of cyanide ever reported in a highly polar solvent.[3,4]

A clear isosbestic point at 414 nm indicates that there is a one-to-one equilibrium reaction between host **1** and cyanide. Job analysis for the binding of host **1** and cyanide also corroborates the 1:1 binding stoichiometry.

To evaluate the binding affinities for a variety of anions, we have carried out UV–vis titration in DMSO. The results are summarized in Table 1. Besides cyanide anion, strongly basic anions ($pK_a > 9$) such as fluoride, phosphate, acetate also show micromolar binding affinities (Entries 1–4), whereas less basic anions ($pK_a < 2$) such as chloride, bromide, iodide, nitrate, and hydrogen sulfate display much lower binding affinities (Entries 6–10). The azide anion with pK_a around 8 shows a moderate dissociation constant of ca. 10^{-5} M (Entry 5). It is

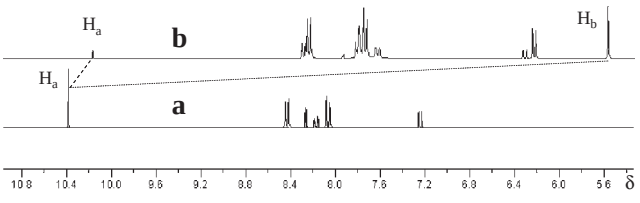


Figure 1. ^{1}H NMR spectral change of **1** upon addition of cyanide anion. (a) **1** only and (b) $[CN^-]/[\mathbf{1}] = 1.5$, where $[\mathbf{1}] = 5.0$ mM in DMSO-d_6 at 25 °C.

Table 1. UV–vis titration data between **1** and various tetrabutylammonium anion guests[a]

Entry	Guest	pK_a in DMSO[b]	K_d/M
1	F^-	15	$3.15(\pm0.74) \times 10^{-6}$
2	$H_2PO_4^-$	NA[c]	$7.26(\pm1.94) \times 10^{-7}$
3	CN^-	12.9	$1.95(\pm0.56) \times 10^{-6}$
4	$CH_3CO_2^-$	12.3	$9.99(\pm2.63) \times 10^{-7}$
5	N_3^-	7.9	$3.60(\pm0.06) \times 10^{-5}$
6	HSO_4^-	NA[c]	$2.33(\pm0.40) \times 10^{-3}$
7	NO_3^-	NA[c]	$<10^{-1}$
8	Cl^-	1.8	$<10^{-1}$
9	Br^-	0.9	$<10^{-1}$
10	I^-	NA[c]	$2.02(\pm0.25) \times 10^{-2}$

[a][**1**] = 10 μM in DMSO at 25 °C. [b]Ref. 9. [c]Not available.

remarkable cyanide operates as a nucleophile while the other strongly basic anions (F^-, $H_2PO_4^-$, and $CH_3CO_2^-$) as bases, (Figure S3) although all of them exhibit micromolar sensitivities.

The phenol proton of host **1** in fact plays a critical role for the binding affinity to cyanide. To elucidate the role of the phenol proton, we have measured ^{1}H NMR spectra with the model compounds, salicylaldehyde and anisaldehyde. Anisaldehyde, which does not have a phenol proton, shows no significant change upon addition of excess cyanide, while salicylaldehyde apparently reacts with cyanide to afford a cyanohydrin as observed in host **1**. This supports the general acid activation of the phenol proton in **1** for the nucleophilic reaction.

Compound **2** without aldehyde function, operating simply as an acid, has shown a weaker binding affinity to the cyanide anion compared to host **1**: $K_d = 1.95\ (\pm0.56) \times 10^{-6}$ (**1**) vs $2.38\ (\pm0.64) \times 10^{-5}$ M (**2**). Host **1** shows ca. 10 times as much enhancement in the binding affinity although both hosts have almost the same pK_a's; pK_a was measured in 2.5% DMSO in water and was found to be 9.47 and 9.55 for **1** and **2**, respectively. Besides basicity, the nucleophilicity of cyanide toward **1** possibly induces additional binding affinity and leads to the observed higher binding affinity.

Cyanide is visually detectable at micromolar concentration. Addition of the cyanide to the 50 μM of **1** in DMSO exhibits a clear color change from light yellow to dark red, while anions with weak basicity induce no color changes. (Figure S5) To evaluate the detection limit of cyanide concentration, we have monitored the color change by increasing the amount of cyanide. The light yellow color of **1** changes into a light red color even in the presence of 0.5 μM of cyanide, indicating the concentration limit of cyanide to be less than 0.5 μM (Figure 2). This means

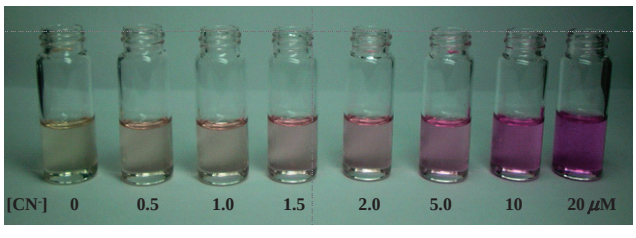


Figure 2. Determination of color detection limit of cyanide by increasing the amount of cyanide, where [**1**] = 10 μM in DMSO was used.

that our colorimetric sensor is operating well in DMSO below the World Health Organization (WHO) cyanide detection level (1.9 μM)[10] and is therefore useful to determine the lethal level (ca. 20 μM) of cyanide in fire victims.[11]

In conclusion, a salicylaldehyde-based azo compound displays a red color upon binding to the biologically toxic cyanide with micromolar sensitivity. The higher sensitivity of **1** toward CN^- is well below the WHO detection level. The higher affinity is caused by the intramolecular hydrogen bond of a phenol proton, which stabilizes the developing anionic character of the intermediate molecule during the nucleophilic attack of a cyanide anion. This nucleophilic character of the cyanide anion can be utilized in the detection of biological toxic cyanide in living systems.

Support of this work from the Korea Research Foundation (KRF-2006-312-C00592) is gratefully acknowledged.

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Published on the web (Advance View) June 5, 2007; doi:10.1246/cl.2007.816