Inorg. Chem. **2008**, *47*, 1264−1266

Inorganic Chemistry
Communication

Specific Colorimetric Detection of Cyanide Triggered by a Conformational Switch in Vitamin B$_{12}$

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Received December 5, 2007

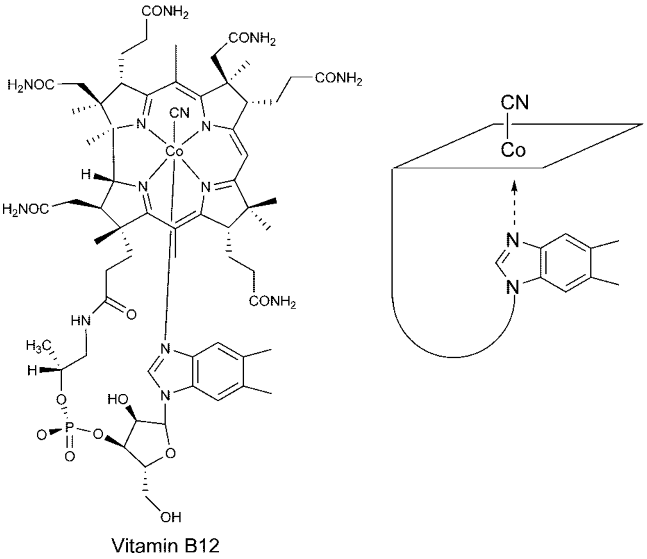
"Base on"/"base off" coordination of the intramolecular bound benzimidazole nucleobase of vitamin B$_{12}$ allows the specific colorimetric detection of millimolar concentrations of cyanide in water. In various competition experiments, it was demonstrated that up to 12 different anions as well as a 1000-fold excess of Cl$^-$ over CN$^-$ do no interfere with the sensor. An 8-fold increased sensitivity in the "naked eye" detection of CN$^-$ was observed when water was replaced by MeOH/H$_2$O (5%) as a solvent.

The extreme toxicity of the cyanide anion and its use in many industrial applications points out the importance to detect this dangerous chemical.[1] The toxicity arises from the binding of cyanide to cytochrome a_3, which inactivates cellular respiration and affects the central nervous system.[2]

Different detection systems including electrometric,[3] fluorometric,[4] and chromatographic[5] techniques have been established to detect free cyanide, but most of them need laborious sample preparation as well as expensive and bulky laboratory systems. Recently, carefully designed synthetic receptors for the *so-called* "naked eye" detection[6] of cyanide have attracted much attention.[4b,c,7]

These systems are based on either metal complexes or organic chromophores and convert the coordination or reaction with cyanide into a visual signal. Their applicability is still limited because of poor water solubility as well as low selectivity, especially in the presence of certain halides.

Scheme 1. Structural Formula of the "Base on" Vitamin B$_{12}$ (Left) and Its Schematic Representation (Right)



The coordination chemistry of vitamin B$_{12}$ (Scheme 1) and its derivatives has been extensively explored, and it was demonstrated that $\pi-\pi^*$ transitions of the tetradentate macrocycle are extremely sensitive to the nature of additional axial ligands.[8]

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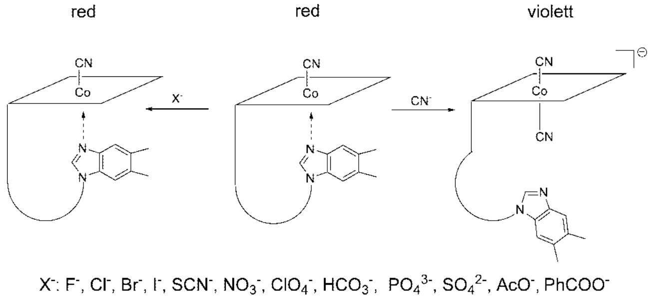
10.1021/ic702368b CCC: $40.75

Scheme 2. Mechanism of the Vitamin B_{12}-Based Cyanide Sensor[a]



[a] CN^- triggers the formation of the "base off" dicyano-B_{12} (right) from "base on" vitamin B_{12} (middle), whereas no decoordination takes place in the presence of other anions X^- (left).

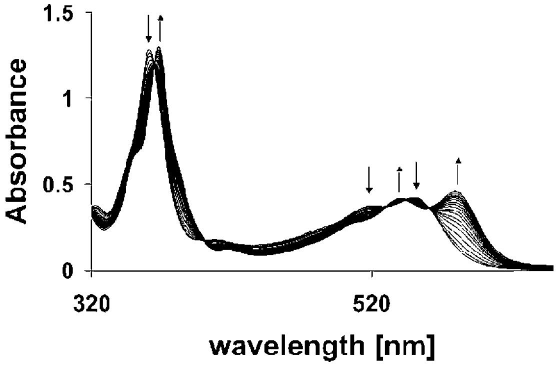


Figure 1. Absorption spectra of a solution of B_{12} (50 μM) upon the addition of increasing amounts of CN^- (0–10 mM) in water ([Hepes] = 20 mM; pH 7.6 ± 0.1) at $T = 22 \pm 2$ °C.

Therefore, special "hydrophobic" cobalt(III) corrin derivatives with one vacant axial coordination side replacing the benzimidazole nucleobase of vitamin B_{12} have already been applied in cyanide sensing.[9] A drawback of these systems is their unselective response toward other coordinating anions in the absence of cyanide.

To the best of our knowledge, unmodified vitamin B_{12} itself has not yet been applied in this context. In the "base on" vitamin B_{12}, the axial positions of the cobalt(III) macrocycle are occupied with cyanide at the β side and an intramolecular coordinated benzimidazole at the λ side (Scheme 1).

We assumed that a cyanide-triggered displacement reaction of the benzimidazole nucleobase to dicyano-B_{12} would improve the specificity in sensing (Scheme 2).

Here, we report that vitamin B_{12} can be applied for the specific visual detection of millimolar cyanide in water.

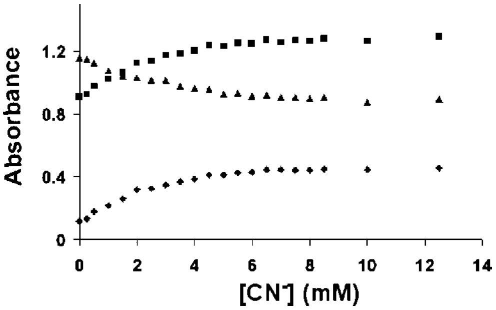


Figure 2. Calibration curves at 580 nm (♦), 368 nm (■), and 360 nm (▲) of the vitamin B_{12}-based CN^- sensor.

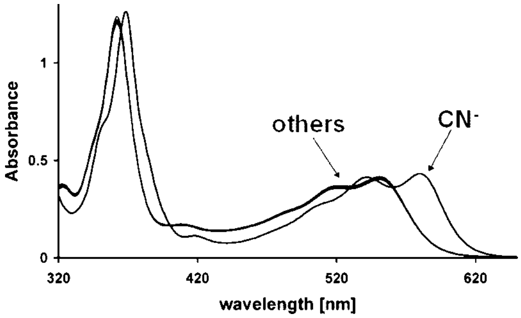


Figure 3. Absorption spectra of solutions of vitamin B_{12} (50 μM) treated with F^-, Cl^-, Br^-, I^-, SCN^-, CN^-, NO_3^-, ClO_4^-, HCO_3^-, PO_4^-, SO_4^{2-}, AcO^-, or $PhCOO^-$ (5 mM) in water ([Hepes] = 20 mM; pH 7.6 ± 0.1) at $T = 22 \pm 2$ °C.

In a first experiment, cyanide (0–10 mM) was added stepwise to a solution of vitamin B_{12} (50 μM) in water ([Hepes] = 20 mM; pH 7.6 ± 0.1) at 22 °C (Figure 1). The color changed from red to violet accompanied by a shift of the absorption bands from λ_{max} = 550, 520, and 361 nm to λ_{max} = 580, 541, and 368 nm. This is due to the decoordination of the benzimidazole nucleobase of B_{12} in its "base on" conformation to the "base off" dicyano-B_{12} form (Scheme 2).[8a,10] Six isobestic points at 561, 543, 531, 400, 364, and 318 nm were observed, indicating a clean conversion without any side equilibrium (Figure 1).

The titration curves were analyzed with the program *Hyperquad2006* to yield a binding constant *K* of 1.8 (±0.6) × 10^2 M^{-1}.[11] This value is slightly lower than that previously reported in a dimethyl sulfoxide/propanol mixture because the anion is probably stronger hydrated.[12] Moreover, calibration curves generated at λ_{max} = 580, 368, and 361 nm demonstrate that the vitamin B_{12}-based sensor is potentially useful for the quantitative cyanide determination in the linear response range between 0 and 1 mM with a detection limit of 0.25 mM (Figure 2).

When we added one of the anions F^-, Cl^-, Br^-, I^-, SCN^-, NO_3^-, ClO_4^-, HCO_3^-, PO_4^{3-}, SO_4^{2-}, AcO^-, or $PhCOO^-$ (5 mM) to a buffered solution ([Hepes] = 20 mM; pH 7.6 ± 0.1) of vitamin B_{12} (50 μM) at room temperature ($T = 22 \pm 2$ °C), the red color of the vitamin B_{12} solution as well as the characteristic absorption bands of vitamin B_{12} in its "base on" conformation (λ_{max} = 550, 520, and 361 nm) remained uneffected, indicating that no ligand displacement took place (Scheme 2 and Figures 3 and 4).

However, after the addition of cyanide (5 mM) to a vitamin B_{12} solution under the same conditions, a color change to the violet "base off" conformation (λ_{max} = 579, 542, and 368 nm) could be easily detected with the "naked eye" within a few minutes (Scheme 2 and Figures 3 and 4). Various

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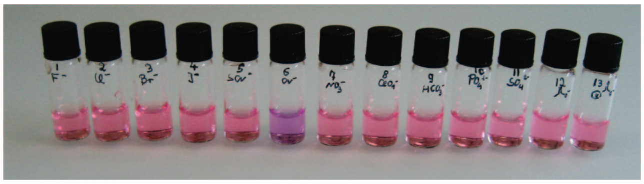

Figure 4. Color of solutions of vitamin B_{12} (50 μM) treated with F^-, Cl^-, Br^-, I^-, SCN^-, CN^-, NO_3^-, ClO_4^-, HCO_3^-, PO_4^{3-}, SO_4^{2-}, AcO^-, or $PhCOO^-$ (5 mM; from left to right) in water ([Hepes] = 20 mM; pH 7.6 ± 0.1) at $T = 22 \pm 2$ °C.

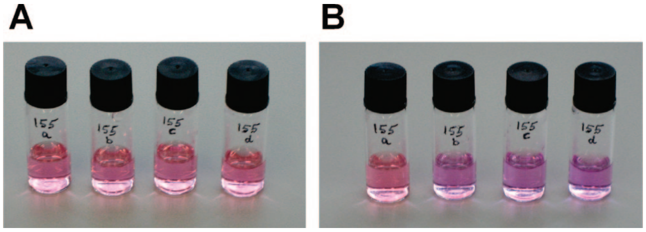

Figure 5. (A) Color of solutions of vitamin B_{12} (50 μM) in the presence of no additional anion (first vial), Cl^- and SCN^- (1 M each; second vial), Cl^- (5 M; third vial), and all 12 anions X^- (5 mM each; fourth vial) (from left to right) in water ([Hepes] = 20 mM; pH 7.6 ± 0.1) at $T = 22 \pm 2$ °C. (B) Color changes observed after the addition of either no anion (first vial) or CN^- (5 mM) (second to fourth vials) to the solutions of part A.

competition experiments were performed to test the specificity of this sensor. At 1 M Cl^- and SCN^- each, the vitamin B_{12} sensor (50 μM) remained in its red-colored "base on" conformation but turned to its violet-colored dicyano form after the addition of CN^- (5 mM; Figure 5).

The same effect was observed in the presence of 5 M Cl^- corresponding to a 1000-fold excess over cyanide (Figure 5).

Moreover, the addition of all 12 anions (5 mM each) besides cyanide did not influence the red color of the vitamin B_{12} sensor, but again after the addition of cyanide, the sensor turned to violet (Figure 5).

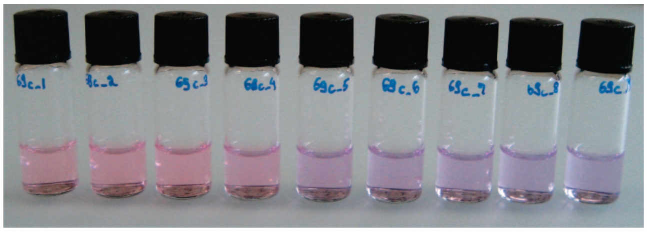

Figure 6. Color of solutions of vitamin B_{12} (20 μM) treated with increasing amounts of CN^- (0, 100, 200, 400, 600, 800, 1000, 1500, and 2000 μM; from left to right) in MeOH/H_2O (5%) at $T = 22 \pm 2$ °C.

Specific sensing of CN^- was also observed at pH 11.8 ± 0.1 ([phosphate] = 20 mM), demonstrating the general applicability of the sensor over a wide range of reaction conditions.[13]

When water as a solvent was replaced by a mixture of MeOH/H_2O (5%), the sensitivity of the vitamin B_{12} sensor (20 μM) in the "naked eye" detection of CN^- could be increased by a factor of 8 to a detection limit of 600 μM (Figure 6) probably because the cyanide is less hydrated and its nucleophilicity is enhanced.

In summary, commercially available vitamin B_{12} is useful for the "naked eye" detection of millimolar CN^- in water and improves the discrimination toward other anions. The mode of operation of this sensor is controlled by its natural benzimidazole trigger of being able to switch between different conformational states.

Acknowledgment. F.H.Z. is grateful to the University of Zürich and R. Alberto for generous support. The author thanks B. Spingler for helpful discussions and M. Jöhri for taking the photographs.

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(13) Data not shown.