A molecular logic gate for the naked-eye detection of glutathione or pyrophosphate with a nickel based bio-sensor

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Abstract An inorganic water soluble Ni(II) complex offers a molecular logic gate for the naked-eye detection of pyrophosphate versus thiol derivatives. Pyrocatechol coordinated to nickel performs a thiol-michael addition click reaction in the presence of thiol derivaties whereas pyrophosphate allows a displacement of the indicator PCV leading to an indicator displacement assay.

1 Introduction

Biological thiols such as (Cys), homocysteine (Hcy) and glutathione (GSH), are essential in biological systems. They play a key role in protein structures and control redox homeostasis. Abnormal levels of biological thiols are implicated in a variety of diseases such as cancers for example with GSH. \textsuperscript{[1]} Hence, thiol containing molecules such as Glutathione (GSH) or cysteine (Cys), aim generally at detoxifying metals according to the literature. \textsuperscript{[2]} \textsuperscript{[3]} \textsuperscript{[4]} For example, GSH -a thiol containing peptides- is produced in human cells for the detoxification of poisonous metals such as cadmium. \textsuperscript{[5]} Even if it is well accepted that GSH detoxify metals, the coordination modes are numerous and differ depending on metals. Moreover, very few modes are reported for precious metals such as Ruthenium or Palladium. \textsuperscript{[6]} Here, we show that nickel complexes, bearing pyrocatechol violet (PCV) as a ligand, can be easily formed in buffered conditions. \textsuperscript{[7]} These blue nickel complexes can be scrutinized using a so-called Indicator Displacement Assay (IDA) \textsuperscript{[8]} to recognize phosphate or thiols containing molecules such as pyrophosphate (PP) or GSH/Cys. The PCV ligand forming the Ni-complex is also a 1,4-michael acceptor, therefore a competition with an indicator displacement assay and a 1,4 - michael addition (1,4-MA) for recognition may take place. We show that, for both cysteine and L-reduced GSH, the 1,4-MA is surprisingly preferred to the IDA, suggesting that thiol containing molecules are more likely to engage a 1,4-MA than a ligand exchange, even in presence of a metal. When in presence of PP, the indicator displacement assay is preferred though. The one-complex two mechanisms possible reaction gives birth to a boolean two-output signal molecular logic gate. \textsuperscript{[9]}

2 Materials and methods

Dissolving pyrocatechol violet (PCV, 250 \textmu M) and nickel sulfate (250 \textmu M) in 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES, 100 mM, pH=7.4) formed [Ni(PCV)], which is blue colored at working micromolar concentrations (Figure 1). By analogy with other reported dinuclear complexe \textsuperscript{[10]} \textsuperscript{[8]}, we hypothesized that the [Ni(PCV)] complex could be used to assemble a receptor for phosphate derivatives, exploiting metal-ligand interactions for selective target recognition. To the best of our knowledge, this mononuclear complex has

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never before been described in the literature as a selective IDA-biosensor for pyrophosphate derivatives (See figure 1). We chose the reported pyrocatecholviolet (PCV), a catechol-type pH-sensitive dye [11], as the chromogenic indicator for the sensor, due to a plethora of successful reports in the literature for various indicator displacement assays. [10] [12] [13] [14] [7, 10, 14] [15] [16] [17] [18]

\[ \text{Figure 1. Mixing nickel sulfate (250 \, \mu M) with pyrocatechol violet in HEPES 0.1 M at pH = 7.4 (250 \, \mu M) offers a blue colored complex (250 \, \mu M) a) Addition to the construct of pyrophosphate (PP, 250 \, \mu M) allows an indicator displacement assay producing a green solution b) Addition of thiol derivatifs (250 \, \mu M to 25000 \, \mu M) to pyrocatechol violet allows a 1.4-michael addition generating a intense yellow solution c) Addition of thiol derivatifs to [Ni(PCV)]^{2+} allows a 1.4-MA procucing same color as reaction c d) } \]

In addition to being a pH-sensitive chromophore, pyrocatechol violet, which is green-yellow color at neutral pH (reported peak at \( \lambda_{\text{max}} = 444 \, \text{nm} \)) changes to blue (reported \( \lambda_{\text{max}} = 624 \, \text{nm} \)) when coordinated to a metal. Therefore, the displacement of the receptor-bound pyrocatechol violet by a phosphate derivativ analyte is communicated visually as well as being readily measured spectrophotometrically (Figure 1).

In this spirit, we screened various phosphate derivatives for detection. The competition assay developed is illustrated schematically in figure 1. The sensing ensemble was prepared by simply mixing PVC and NiSO\(_4\) in a 1:1 molar ratio in an aqueous solution of 100 mM HEPES buffer pH = 7.4, resulting in a blue complex (peak with \( \lambda_{\text{max}} = \text{nm} \)). The absorbance of the construct is depending on the concentration according to the Beer-Lambert law, [Ni(PCV)] at 250 \, \mu M has an absorbance of X at a \( \lambda_{\text{max}} = XX \, \text{nm} \)

We found that only pyrophosphate (PP) was best appropriated for the displacement over other anions and in particular phosphate. We obtained a green-yellow colour after detection at micromolar concentrations with PP. The detection limit of PP was: x and the affinity constant: x that is close to similar descriptions in the literature.
In addition, PCV is a 1,4-michael acceptor (See fig 1). We therefore investigated the possibility of an addition of thiol derivatives on PCV. After a small screen we found that cystein was the privileged molecule for a 1,4-michael addition on pyrocathocol violet. Homocystein didn’t reacted with PCV and suggested that indeed the thiol part on the molecule is involved in the reaction. Remarkably, L-reduced glutathione was detected with this system. The association constants of thiol derivatives is resumed in table 1, GSH has an association constant of: X . Albeit the $K_{as}$ is small, it is similar to the concentration of GSH encountered in cells and could therefore be used to measure the internal concentration of GSH in vivo as described by various groups, and especially: Liu and coworkers [19] and Umezawa and coworkers [20].

With both reactions in hands we decided to extend the concept of simultaneous detection of analytes leading to a molecular logic gates (See figure 3). We therefore decided to test [Ni(PCV)] for the detection of pyrophosphate or for the detection of Cys/GSH. As described, pyrophosphate generated a displacement of PCV whereas surprisingly Cys/GSH performed a 1,4-michael addition on the indicator. This is in opposition with the literature that pretend the high affinity of GSH for metals. We would have therefore anticipated a ligand exchange. In order to challenge our new observation, we decided to investigate various metal-PCV based complexes such [Zn(PCV)], [Cu(PCV)], [Ce(PCV)]$^{2+}$ for the addition of Cys/GSH. Same trends where observed. This suggests that in the presence of various metals, the 1,4-michael addition is prefered to the displacement of the cathecol based ligand.

Figure 2. The mix between NiSO$_4$ (250 $\mu$M) with pyrocathcol violet (PCV) at 250 $\mu$M forms [Ni(PCV)] that is blue colored with a $\lambda_{abs}$ = nm addition of PP (250 $\mu$M) to the inorganic complex generates a decrease in absorbance at $\lambda_{obs}$ = nm and an increase in absorbance at $\lambda_{obs}$ = nm a). Addition of excess of GSH changes completely the spectra of PCV and generates an increase in the absorbance at $\lambda_{obs}$ = nm and a strong decrease in absorbance at $\lambda_{obs}$ = nm whereas addition of pyrophosphate doesn’t offer this remarkable color change b).
In summary, our observations allow to conclude that:

i) [Ni(PCV)] can be used for the naked-eye detection of pyrophosphate selectively in solution at micromolar conditions (See Fig.2).

ii) PCV can be used as a 1,4-michael acceptor for thiol based molecules in solution changing color to intense yellow (See figure 2). The association constant between PCV and R-SH is depending on the molecule and albeit high, in the range of the concentration of GSH concentration in cells (See table 1).

iii) [Ni(PCV)] can be used for the simultaneous detection of pyrophosphate versus Cys/GSH. Pyrophosphate allows an indicator displacement whereas Cys/GSH allows a 1,4-Michael addition. The one complex two detection give birth to a molecular logic gate (See figure 2 and 3).

iii) On various metal-PCV based complexes the 1,4-michael addition in preferred in contrary to ligand exchange. It is contrasts in this situation with literature descriptions.

3 Conclusion

In summary, we have created a colorimetric nickel based sensor able to detect selectively pyrophosphate of thiol based molecules. The complex changes color from blue to pale yellow-green in the presence of pyrophosphate whereas it changes to intense orange-yellow colors in the presence of Cys/GSH. The one complex two reaction give birth to a molecular logic gate sensors. The complex is able to detect selectively...
Figure 4. Boolean molecular logic gate (a) Truth table of the molecular logic gate sensor [Ni(PCV)], input is both PP (A) or -SH (B) whereas output is both green (F) or yellow color (G) (b)

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