

Vanadium Oxide Nanoparticle-Based Cysteine Optical Sensor

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Abstract: We report on the interaction of vanadate nanoparticles with cysteine in biological molecules. The colloid undergoes a color change upon reaction with cysteine-containing molecules. This selective interaction could be used as an efficient cysteine-optical sensor.

1. Introduction

Cysteine (Cys) is a nonessential amino acid present in most proteins. It has numerous biological functions and is a major antioxidant in animal cells [1]. We report on the interaction between Cys and vanadate nanoparticles (VNP) produced through laser ablation in liquids synthesis. Laser ablation is a green technique that allows the synthesis of colloids in pure water without the need of other chemicals [2]. We found that VNP undergo a color change upon reaction with cysteine containing molecules; in addition, a broad absorption band, the so called intervalence band, appears near the infrared. This intervalence band of the optical absorption spectra shows capability for quantitative cysteine sensing in the μM range in biological macromolecules.

Detailed UV-Vis absorption spectra and dynamic light scattering (DLS) measurements were done to investigate the detection of cysteine in large biological molecules. Tests included cytoplasmic repetitive antigen (CRA) and flagellar repetitive antigen (FRA) proteins of the *Trypanosoma cruzi* protozoa, as well as the capsid p24 proteins from Human Immunodeficiency Virus type 1 (HIV-1) and type 2 (HIV-2). Detailed NMR measurements for hydrogen, carbon and vanadium nuclei show that cysteine in contact with the vanadate loses hydrogen of the sulphhydryl side-chain, while the vanadate is reduced. The subsequent detachment of two deprotonated molecules to form cystine and the slow return to the vanadate complete the oxidation-reduction cycle. Therefore, the vanadate acts as a charge exchanging catalyst on cysteine to form cystine. The interaction of VNPs with cysteine is sensitive and completely selective, and could be used as an efficient optical sensor for cysteine containing molecules.

2. Experimental Details

A Q-switched Quantronix Model 117 Nd:YAG laser (1kHz, 200ns) at 1,064 nm was used in the experiments [3]. The laser beam was focused with a 50mm lens on a vanadium target (Williams Advanced Materials). The target was placed 2 mm under bidistilled water, and irradiation time was of 5 min, leading to a yellowish suspension of 5.4 mM vanadium in water. The nanoparticle size distribution was measured using combined techniques such as DLS, TEM, and AFM. The VNP interaction with cysteine was measured using linear optical absorption techniques (UV-Vis-NIR) and Nuclear Magnetic Resonance (NMR).

The aminoacid Cys and the tripeptide GSH, composed by three aminoacids Cys, glutamic acid, and glycine (Sigma-Aldrich Corporation 99 %) were dissolved in bi-distilled water and added to the yellowish nanoparticle suspension. *Trypanosoma cruzi* genes were cloned in lambda gt11 and screened with an anti-trypanomastigote antiserum. Two clones were selected in view of their reactivity with human chagasic sera. One clone encodes a flagellar repetitive antigen (FRA), whereas the other corresponds to a cytoplasmic repetitive antigen (CRA) [1]. The analyses were carried out also using the p24 capsid proteins of the Human Immunodeficiency Virus type 1 (HIV-1) and type 2 (HIV-2), whose aminoacid sequences were retrieved from the National Center for Biotechnology Information (NCBI). The protein production was carried out in bacterial system, and the resultant antigens were purified through a single step of Ni-NTA affinity chromatography, followed by quantification through densitometry assays. It is important to point out that the HIV-2 p24 molecule contains twice as many Cys building blocks as HIV-1 p24, whereas Cys is absent in the CRA and FRA molecules.

3. Results

The addition of Cys to the yellowish V_2O_5 nanoparticle suspension shifts the light absorption from the blue to the UV region and the suspension gets bluish transparent, while pure Cys absorbs light only in the far UV region. Fig. 1 shows the typical spectra, which demonstrate a strong concentration- dependent optical absorption characteristic in the 350–480 nm region.

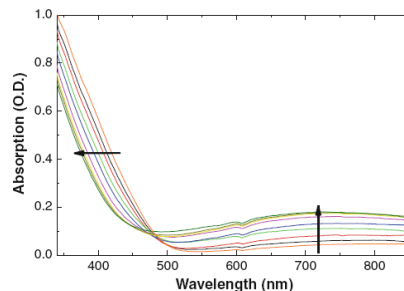


Fig. 1. Absorption spectra of the nanoparticle suspension with increasing Cys addition. The arrows indicate increasing Cys concentration.

In addition to this color shift, a broad absorption band appears at longer wavelengths. This absorption band has an intensity which also depends on the Cys concentration in the suspension. This part of the spectra was analyzed in more detail, using several Cys, GSH, CRA, FRA, HIV-1, and HIV-2 additions to the V_2O_5 nanoparticle suspension. The plot of the optical absorption at the 720 nm wavelength versus Cys/GSH/protein concentration is shown in Fig. 2, and a linear relationship can be observed.

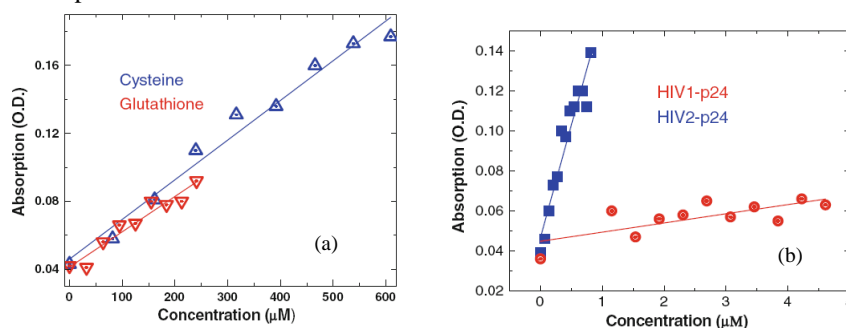


Fig. 2. Optical absorption at 720 nm: (a) versus Cys (upright triangles) or GSH (inverted triangles) concentration; (b) versus HIV1-p24 and HIV2-p24 concentration in the suspension

The CRA/FRA proteins do not contain Cys in their structure, and we could detect no changes in the optical properties by adding both these proteins to the V_2O_5 nanoparticle suspension, which indicates its selectivity to Cys detection.

4. Discussion and Conclusions

We have tried to reproduce this optical sensor by using commercial V_2O_5 powder in water, to no avail. In this regard, our NMR results also indicate that the (tetragonal) V_2O_5 nanoparticles produced by laser ablation are not formed by its common orthorhombic form. This may be an indication that not only nanoparticle size but also the laser ablation synthesis itself may be essential for the reported spectral change. The interaction of VNP with cysteine is sensitive and completely selective, and could be used as an efficient optical sensor for cysteine containing molecules. For instance, it could find applications in the detection of folding/unfolding of proteins.

5. References

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