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Inorganic-bimolecular hybrids based on polyoxometalates: Intrinsic oxidase catalytic activity and their application to cancer immunoassay

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ABSTRACT

A series of inorganic-biomolecular hybrids $FA_nPMo_{12-n}V_nO_{40}$ (n = 1, 2, and 3, abbreviated as $FAPMoV_n$, FA represents folic acid) had been synthesized through self-assembly of bioactive molecules folic acids and phosphovanadomolybdates. These folate-functionalized hybrids had been proved to possess unique oxidase-like activity in the oxidation of dyes, which can catalyze oxidation of the peroxidase substrate 3,3′,5,5′-tetramethylbenzidine (TMB) by air to form a blue color in aqueous solution. Among all hybrids, $FAPMoV_3$ performed best. Based on their oxidase property, an approach instead of traditional horseradish peroxidase (HRP)-labeled assay for the visual detection of cancer tumors by $FAPMoV_3$ had been built up. $FAPMoV_3$ exhibited fast response and high efficiency in colorimetric immunoassay of cancer cells at a neutral pH condition.

1. Introduction

Polyoxometalates (POMs) are oxo-clusters of early transition metals in the highest oxidation state, namely Mo(VI), W(VI) or V(V), which represent an increasingly important class of potential processes for the oxidation of numerous organic substrates in the presence of either molecular oxygen, H_2O_2 or other donors [1–4] even under the mild conditions. By this oxidative property, POMs had been used as catalysts in one- or two-electron-transfer oxidation of organic substrates into the corresponding compounds [5], which was recognized as inorganic oxidase. Among all POMs in oxidative catalysis, phosphovanadomolybdate compounds, $PV_nMo_{12-n}O_{40}^{(3+n)-}$ (n = 1–6, but especially 2) were most active species for the application as oxidase catalysts in organic synthesis [6,7]. These years witnessed the development of catalytic oxidation of organic substrates based on $PMo_{10}V_2O_{40}^{5-}$, and less attention had been paid for the investigation of other vanadium species. Recently, we have reported that $PMo_{10}V_2O_{40}^{5-}$ is a potential catalyst for the oxidation of 3,3′,5,5′-tetramethylbenzidine (TMB) to its oxidative form with blue color, which could be used as an indicator for colorimetric immunoassay of the cancer cells [8]. In the past decade, there has been a growing interest in exploring the biological activity of POMs in antiviral, antibacterial, and antitumor arease [9–15]. Our work lied in developing efficiently colorimetric immunoassay for the cancer cells or other biological molecules, which gave more chance for POMs in biological applications. In the present paper, we reported on an extension of our research work. A series of phosphovanadomolybdate compounds, $H_{3+n}PV_nMo_{12-n}O_{40}$ (n = 1, 3) had been studied in order to enrich the oxidative catalysis and distinguish the difference of structure–function-properties between the species with different number of vanadium.

The unique properties of nanomaterials have dramatically expanded the field of nanotechnology in wide of applications and industry sectors. Self-assembly is emerging as a powerful, and bottom-up approach for the fabrication of novel functional nano- or biomaterials [16]. It is a new field that needs to be developed by varying guest materials ranging from small molecules, nanoscale materials to endow novel functions such as catalysis, biosensors and so on. Many bioactive molecules had been selected as building blocks for self-assembly. And polyoxometalates were used as possible inorganic functional groups for the fabrication of such hybrids with bioactive molecules such as peptide and folate [8,17]. These biological molecules functionalized POMs had already been used in immunoassay reported by our group [8,18].

As a continuation, we synthesized different phosphovanadomolybdate $PV_nMo_{12-n}O_{40}^{(3+n)-}$ (n = 1–3)/folates ($FAPMoV_n$) hybrid

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nanoparticles using assembly method. The extension of such investigations should provide further insight into the structure–property-function relationships that exist in such nanostructure bioactive organic–inorganic hybrids. In addition, more active species in colorimetric immunoassay need to be developed. The results showed that folate-functionalized $FAPMoV_3$ was more active and could be used in low concentrations. It was proved to be more inexpensive, easier preparation, and more efficient in a wide range of new potential applications in biotechnology, environmental chemistry, and medicine.

2. Methods and experiments

2.1. Chemicals and materials

All solvents and chemicals were used as obtained from commercial supplies. The purchased chemicals (analytical grade) were used without further purification. Folate acid was purchased from Huishi Biochemical Reagent Company, China. 3,3′,5,5′-tetramethylbenzidine (TMB) was purchased from j&kchemica, Beijing. The heteropolymolybdates $H_{(3+n)}[PMo_{12-n}V_nO_{40}]$ (n = 1–3) were synthesized according to a previous method [19] and identified by IR spectroscopy.

2.2. Apparatus and instrumentations

UV–vis spectra (200–600 nm) were recorded on a Cary 500 UV–vis-NIR spectrophotometer. Elemental analysis was carried out using a Leeman Plasma Spec (I) ICP-ES and a P-E 2400 CHN elemental analyzer. FTIR spectra (4000–400 cm^{-1}) were recorded in KBr discs on a Nicolet Magna 560 IR spectrometer. XRD patterns of the sample were collected on Japan Rigaku D_{max} 2000 X-ray diffractometer with Cu Kα radiation (λ = 0.154178 nm). The ^{31}P MAS NMR measurements were obtained using a Bruker AM500 spectrometer at 202.5 MHz. TEM image was measured on JOEL JEM-2010 microscope. Dynamic light scattering (DLS) was employed in order to study the size of the $FAPMoV_n$ spheres in the Microtrac S3500 Particle Analyzer in terms of the hydrodynamic radius. Pictures of cells were taken on inverted fluorescence microscope (1X2-I LL100). The conductivity versus concentration was determined using Conductivity Meter (DDS-11A).

2.3. Preparation of the FA functionalized $PMoV_n$ (n = 1–3)

0.057 g FA was dissolved absolutely in 100 mL of acetic acid (36%). Then excessive $H_{3+n}PV_nMo_{12-n}O_{40}$ (n = 1–3) was added slowly under vigorously stirring. Immediately, yellow precipitates were formed, and further stirred the mixture until no more precipitate was formed. Finally the solid was filtered and dried in the air.

2.4. Immunoassays

All cell lines were kept at 37 °C, 5% CO_2 in a humidified incubator. Cells were plated in 96 well plate at about density of 5000 cells per well and fixed using polyformaldehyde after 24 h incubation, then treated with 5.0 μM $FAPMoV_n$ nanoparticles, which were allowed to incubate for 4 h. 0.04 mM of TMB was added and cells were washed immediately with redistilled water. Only with 1 min, cells were observed under the inverted fluorescence microscope.

2.5. Oxidase-like activity studies

1 mg of $FAPMoV_n$ (n = 1–3) samples were added to 5 mL of TMB solution (0.08 mM) and the absorbance was determined after 1 min at 652 nm using UV–vis spectroscopy. For time-dependent studies of the $FAPMoV_n$ oxidation of TMB, 1 mg of $FAPMoV_n$ was added to 5 mL of TMB solution (0.04 mM) and the absorbance was determined every 30 s. For pH-dependent studies of the $FAPMoV_n$ oxidation of TMB, 1 mL of TMB (0.04 mM) was mixed with 4 mL citrate buffer with pH from 3 to 7. Then 1 mg $FAPMoV_n$ was added to above citrate buffer and the absorbance were monitored every 1 min at 652 nm on UV–vis spectroscopy.

The oxidative efficiency was represented as a final absorbance value of oxidative form of TMB, which is defined as the oxidative degree of TMB.

3. Results and discussion

3.1. Characterization of the as-prepared FA functionalized $FAPMoV_n$ (n = 1–3)

The FTIR spectra of FA, $PMoV_n$ and $FAPMoV_n$ are shown in Fig. S1. Peaks ranging from 600 to 1100 cm^{-1} corresponding to the Keggin structural vibrations, can be distinguished easily. This indicated that $FAPMoV_n$ all maintain the Keggin structure after assembly. Peaks at 1605 and 1694 cm^{-1} were due to the —N═C— and the C—N stretching bands from FA molecules, respectively, indicating the existence of FA in the assembly hybrids. After the combination of FA and $PMoV_n$, there are obvious shifts of the ν as Mo-O_d, ν as Mo-O_b and ν as Mo-O_c vibrational stretches. Meanwhile, C—N and —O═C— the stretching bands also changed. The above results demonstrated that firstly, the frame structure of POMs were well kept, and secondly, strong binding interactions occurred between $PMoV_n$ and FA molecules [20,21]. The XRD patterns of FA, $PMoV_n$ and the hybrid spheres are shown in Fig. S2. For the $FAPMoV_n$ assemblies, the diffraction peaks at 26.59, 27.56, and 26.50 can be regarded as the feature of the $PMoV_n$ respectively and almost all the diffraction peaks of $PMoV_n$ can be found in the XRD patterns. This further proved that $FAPMoV_n$ all maintained the Keggin structure after assembly. The ^{31}P MAS NMR spectrum of $PMoV_n$ (n = 1–3) was recorded (Fig. S3). It can be seen that signal peak at 4.301, 4.633 and 4.12 ppm for $PMoV_1$, $PMoV_2$ and $PMoV_3$, respectively. The content of each element in the $FAPMoV_n$ was determined by ICP-ES and P-E 2400 CHN elemental analyzer. The measured values: C, 17.15%; H, 1.55%; N, 7.35%; P, 1.15%; Mo, 39.65%; V, 1.90%, respectively, in $FAPMoV_1$ hybrid, giving the formula as $(FA)_4PMo_{11}V_1O_{40}$. The measured values: C, 17.43%; H, 1.58%; N, 7.50%; P, 1.20%; Mo, 36.63%; V, 3.88%, respectively, in $FAPMoV_2$ hybrid, giving the formula as $(FA)_5PMo_{10}V_2O_{40}$. The measured values: C, 17.74%; H, 1.60%; N, 7.62%; P, 1.20%; Mo, 33.56%; V, 5.98%, respectively, in $FAPMoV_3$ hybrid, giving the formula as $(FA)_6PMo_9V_3O_{40}$.

The TEM images of $FAPMoV_n$ (Fig. 1) at 5 d demonstrated that the assemblies were colloidal spheres with diameters ranging from 50 nm to 80 nm. In the dynamic light scattering (DLS) measurement (Fig. S4), the average hydrodynamic diameters of the spherical structures were around 20–50 nm, 50–100 nm, and 80–150 nm corresponding to $FAPMoV_3$, $FAPMoV_2$, and $FAPMoV_1$, respectively. The plots of the specific conductivity versus the concentration of $FAPMoV_n$ showed a break point of nearly two straight-line portions giving the critical micelle concentration (CMC) of $FAPMoV_1$, $FAPMoV_2$ and $FAPMoV_3$ as 8.4×10^{-4} M, 8.7×10^{-4} M and 9.1×10^{-4} M (Fig. S5), respectively. This was also the evidence of the formation of micelles of folate-functionalized POM clusters spheres in aqueous media.

3.2. The morphology evolution of $FAPMoV_2$ from vesicles to tubular aggregates for different storage durations

Folic acid is similar to an amino acid (Fig. S6), and has been successfully used as building blocks to fabricate POMs nanomaterials