Microchimica Acta (2018) 185: 7
https://doi.org/10.1007/s00604-017-2552-1

ORIGINAL PAPER

Layered vanadium(IV) disulfide nanosheets as a peroxidase-like nanozyme for colorimetric detection of glucose

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Received: 29 July 2017 / Accepted: 9 November 2017 / Published online: 2 December 2017

Abstract

The authors have discovered that vanadium disulfide (VS_2) nanosheets, synthesized by a hydrothermal method, exert stable peroxidase-like activity. The catalytic activity, with H_2O_2 as a cosubstrate, follows Michaelis-Menten kinetics and varies with temperature, pH value and H_2O_2 concentration. Two-dimensional VS_2 sheets acting as peroxidase (POx) mimics can replace horseradish peroxidase due to their availability, robustness, and reusability. The POx-like activity of VS_2 sheets was exploited to design a colorimetric glucose assay by using 3,3′,5,5′-tetramethylbenzidine as a substrate and by working at an analytical wavelength of 652 nm. The assay covers the 5 to 250 μM glucose concentration range with a 1.5 μM detection limit. It was applied to the analysis of glucose in fruit juice. In our perception, the peroxidase-like nanozyme out of the family of transition metal dichalcogenides presented here has a wide scope in that it may stimulate promising biocatalytic applications in biotechnology and analytical chemistry.

Keywords Food analysis · Nanocatalysts · Optical · TMB oxidation · Transition metal dichalcogenides

Introduction

Natural enzymes, as traditional efficient and substrate-specific biocatalysts, have been practically applied in food processing, chemical industry, biomedical and biochemistry fields for decades [1]. Whereas, enzymes are usually composed of proteins (or RNAs), thus these high-cost biocatalysts are vulnerable to inactivation unless in the mild condition. To address these drawbacks, artificial enzymes have been developed as robust alternative for natural enzymes. The development of nanotechnology brings great power and application prospect to the research of enzyme mimetics, due to the intrinsic properties of nanomaterials such as large surface-to-volume ratio, high stability, low cost, biocompatibility, and tunable catalytic activity [2–4]. Up to date, many functional nanoscale materials (metal-, metal oxide-, carbon based- nanostructures, etc.) have been reported to imitate the catalysis of natural enzymes, playing key role in biosensor design and biosystem modulation. Developing novel biocatalytic-active nanomaterials (nanozymes) is currently forefront research in inorganic, biological and analytical chemistry.

Nanozymes with two-dimensional (2D) structure such as graphene oxide (GO) [5, 6], possessing high surface-to-volume ratio, can achieve a high load of catalytic substrate on a single nanosheet and provide the superiority of surface engineering strategies. Inspired by the success of GO in nanozyme research, graphene-like two-dimensional layered materials have attracted extensive attention in biocatalysis and biomedicine [7–9]. Vanadium disulfide (VS_2) nanosheets, a representative of the 2D transition metal dichalcogenides (TMDs), have been star nanomaterial in electrochemistry [10], catalysis [11], and energy storage fields [12], due to their

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00604-017-2552-1) contains supplementary material, which is available to authorized users.

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interesting properties gifted by unsaturated d-orbitals of transition metals. Coincidentally, vanadium can be found in many natural enzymes of biological systems. Generally vanadium is in close association with the active center or the cofactor in enzymes such as vanadium chloroperoxidase [13], vanadium bromoperoxidase [14], and vanadium nitrogenase [15]. Linking together the unique properties of 2D VS_2 nanosheets and the richness of vanadium in natural enzymes, nanoscale VS_2 is of great possibility to exert enzyme-like activity, switching on the access of VS_2 to biocatalytic applications. However, to our knowledge the enzyme-like biocatalysis of VS_2 has never been investigated before.

Diabetes mellitus is one of the most globally prevalent diseases, resulting in increasing disability, reduced life expectancy, and enormous healthcare expenses for almost every community [16]. Glucose is a marker for the diagnosis of diabetes, and excessive glucose intake is an important issue concerning the induction or exacerbation of diabetes [17]. Quantitative analysis of glucose in food samples can provide dietary guidelines for diabetic patients. Therefore, novel detection methods are demanded for detecting glucose in food. Among the detection methods for glucose, colorimetric glucose assays using nanozymes are particularly attractive because of their simple operation, low cost, and fast visual signal readout by the bare eye [18].

In this work, we report that layered VS_2 nanosheets, one of the common TMDs, exhibit intrinsic peroxidase-like activity. They can catalyze the oxidation of a substrate in the presence of H_2O_2 to produce a colored reaction product. Moreover, VS_2 nanosheets as POxX-like biocatalysts are demonstrated to exhibit good catalytic properties, stability, and reusability, rivaling many other peroxidase mimetics and natural horseradish peroxidase (HRP). The biocatalytic mechanism of VS_2 POx mimics is also investigated in this work. Finally thanks to these findings, the novel use of such two-dimensional VS_2 as readily available nanocatalysts is demonstrated for sensitive colorimetric detection of glucose in fruit juice.

Material and methods

Chemical and materials

Ammonium metavanadate (NH_4VO_3), thioacetamide (TAA), glucose, fructose, maltose, lactose, and sucrose were purchased from Aladdin chemistry Co. Ltd. (Shanghai, China, http://www.aladdin-e.com/). Glucose Oxidase (GOx, from Aspergillus Niger), 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), 3,3′,5,5′-tetramethylbenzidine (TMB), o-phenylenediamine (OPD), and Glucose (HK) Assay Kit (GAHK20) were purchased from Sigma Aldrich (Shanghai, China, http://www.sigmaaldrich.com/china-mainland.html). NH_3•H_2O, HAc, NaAc, NaOH and anhydrous ethanol were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China, http://www.sinoreagent.com/). Commercial apple juice and orange juice (Minute Maid, Coca-Cola Co., http://www.minutemaid.com/) were purchased from local supermarket. All of these reagents were analytical grade and used as received. Ultrapure water (18.2 MΩ·cm) produced by a Milli-Q system was used throughout this work.

Instruments and characterizations

Field emission scanning electron microscope (SEM) image was taken by an S-4800 (Hitachi, Japan, http://www.hitachi.com/). Powder X-ray diffraction (XRD) patterns were obtained using a powder diffractometer (Bruker D8 Advanced Diffractometer System, Germany, http://www.bruker.com/) with a Cu Kα (1.5418 Å) source. Raman spectra were recorded by a micro-Raman spectrometer (i-Raman Plus, B&W TEK Inc., USA, http://www.bwtek.com/). The UV-vis spectra were measured with a UV-2550 spectrophotometer (Shimadzu, Japan, http://www.shimadzu.com/). All pH measurements were performed with a PB-10 digital pH-meter (Sartorius, Germany, https://www.sartorius.com/) with a combined glass-calomel electrode.

Synthesis of VS_2 nanosheets catalysts

VS2 nanosheets were obtained through a hydrothermal method. Briefly, 8 mmol NH_4VO_3 was dissolved in 72 mL NH_3•H_2O aqueous solution ($V_{NH3•H2O}$: $V_{deionized\ water}$ is 1:11). Note that NH_4VO_3 should be completely dissolved in the solution before the next step. Then, 40 mmol thioacetamide (TAA) was added under magnetic stirring and this mixture was kept stirring for 30 min with the formation of a dark black solution. Subsequently, the solution was transferred into an autoclave and incubated at 180 °C for 24 h. After thehydrothermal treatment, fresh black precipitates were separated by centrifugation, and washed with deionized water and ethanol thoroughly for several times, respectively. The final product was dried at 40 °C in vacuum overnight, yielding the black powder samples denoted VS_2 product.

Peroxidase-like catalysis of VS_2 nanosheets

The time-dependent kinetics of VS_2 nanosheets was studied as follows: A reaction system with different concentration of VS_2 (from 0 to 80 μg·mL^{-1}), 20 mM H_2O_2 and 0.5 mM TMB was monitored using a UV-2550 spectrophotometer in time scan mode at 652 nm right after all of the reagents were mixed. The operation of the leaching experiment is the same as the above, except that the VS_2 solution is replaced with leaching solution. PH test was carried out at room temperature and the pH value of the reaction buffer was adjusted in the

range of 2.0–7.0 by the addition of HCl or NaOH. Temperature test was conducted in acetate buffer (pH 4.0) in the range of 4–70 °C. The re-utilization experiment was carried out using a scale-up reaction system (1 mg VS_2 used) and repeated the operation under identical reaction conditions. The ROS scavenging assays were carried out by adding different concentration of scavenger into the reaction system (50 μg·mL^{-1} VS_2, 20 mM H_2O_2, and 1 mM TMB) and the UV-vis absorption was measured after 15 min incubation.

Kinetic study of VS_2 as peroxidase mimetics

Unless otherwise stated, steady-state kinetic assays were carried out under standard reaction conditions (50 μg·mL^{-1} VS_2 nanosheets at room temperature in pH 4.0 acetate buffer) by varying concentrations of TMB at a fixed concentration of H_2O_2 or vice versa. All the reactions were monitored in time scan mode at 652 nm using a Shimadzu UV-2550. Catalytic parameters were determined by fitting the absorbance data to the Michaelis-Menten equation (Eq. 1).

$$V = \frac{v_{\max}[S]}{K_m + [S]} \quad (1)$$

The Michaelis-Menten equation describes the relationship between the rates of substrate conversion by an enzyme and the concentration of the substrate. In this equation, v is the rate of conversion, v_{max} is the maximum rate of conversion, [S] is the substrate concentration, and K_m is the Michaelis constant.

H_2O_2 and glucose colorimetric detection assays

The H_2O_2 detection assay was conducted by adding increasing amount of H_2O_2 into the reaction system (50 μg·mL^{-1} VS_2 and 1 mM TMB in 0.1 M acetate buffer, pH 4.0) and incubating for 10 min at room temperature. For the glucose calibration, 5 μL of 40 mg·mL^{-1} glucose oxidase (GOx) were mixed with 100 μL phosphate buffered solution (5 mM, pH 7.0) containing different amount of D-glucose and then incubated at 37 °C for 45 min. Subsequently, the glucose reaction solution was added into 400 μL acetate buffer (0.1 M, pH 4.0) containing 1 mM TMB and 50 μg·mL^{-1} VS_2 nanosheets. After incubated at room temperature for another 10 min, the reaction solution was monitored by the UV-vis spectrophotometer. For glucose determination in fruit juice samples (apple juice and orange juice), the juices were firstly centrifuged at 12,000 rpm (7727 rcf) for 40 min to remove the precipitates. Then the supernatants were diluted 100-fold using 0.1 M NaAc buffer (pH 4.0) and stored overnight at room temperature before use. As a comparison, the glucose content in juice samples was also determined by a Glucose (HK) Assay Kit (Sigma Aldrich).

Results and discussion

Structure characterization of VS_2 nanosheets

As schematically illustrated in Fig. 1a, layered VS_2 nanosheets were synthesized via a hydrothermal method, using ammonium metavanadate (NH_4VO_3) and thioacetamide (TAA) as precursor materials. The XRD pattern of the VS_2 nanosheets (before catalysis) is presented in Fig. 1b, in which all characteristic peaks are consistent with the standard card of VS_2 (JCPDS NO. 89–1640). Figure 1c shows the Raman spectrum of the VS_2 (before catalysis) in the range of 100–1100 cm^{-1}. As reported previously, the Raman bands occurring at 140.4, 192.0, 282.0, 406.6, 687.8, and 993.2 cm^{-1} are assigned to the rocking and stretching vibrations of V–S bonds or their combination [19]. As the SEM image in Fig. 1d shows, the as-prepared VS_2 exhibit nanosheet-like morphology with a diameter of 3–6 μm and a thickness of 50–100 nm. As typical two-dimensional nanostructures, VS_2 nanosheets have great application potential in biocatalysis by employing surface engineering strategies, like their analogues (MoS_2 and WS_2) in TMDs family [20–22]. Finally, the chemical maps of the constituent elements V and S were obtained using the energy dispersive X-ray spectroscopy (EDX) technique. Figure 1e shows that V and S elements are homogeneously distributed with the ratio of about 1: 2. The structure characterization results above indicate the successful synthesis of two-dimensional layered VS_2 nanosheets.

Catalytic properties of POx-like VS_2 catalysts

Firstly we studied the catalytic oxidation ability of VS_2 toward peroxidase substrate (ABTS, TMB, and OPD) to evaluate their availability as peroxidase mimics. As showed in Fig. 2a, negligible change can be seen in the UV-vis spectra (The dash lines) when VS_2 was incubated only with peroxidase substrate. However, on addition of H_2O_2, colored oxidation products are formed that have characteristic absorption peaks (oxTMB: 652 nm, oxABTS: 420 nm; oxOPD: 450 nm). The results clearly demonstrate that vanadium disulfide can simulate the catalysis of horseradish peroxidase (HRP). TMB was used as the substrate in subsequent studies because of its acceptable signal generation under catalytic oxidation.

Using the oxidation of TMB by H_2O_2 as a model reaction, the time-dependent kinetics of VS_2 in different concentrations was monitored. As shown in Fig. 2b, the absorbance of oxTMB increases steadily with the increasing VS_2 nanosheets and the time past. This indicates the catalytic reaction is dependent on VS_2 concentration and time. Subsequently, the following catalytic conditions were optimized: (a) reaction pH value; (b) temperature. Respective data and illustrations are given in Fig. S1 and Fig. S2. For simplicity, 10 min reaction time, 50 μg•mL^{-1} VS_2, and mild catalytic conditions (pH

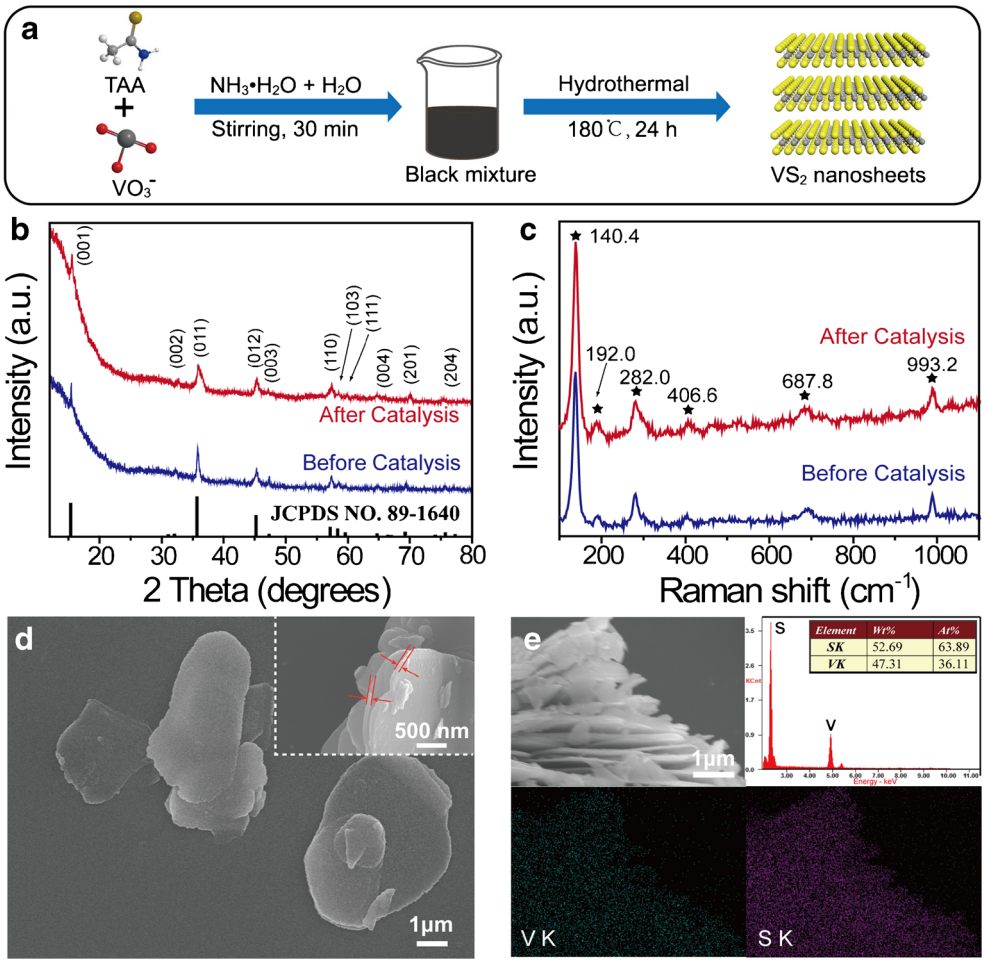


Fig. 1 Synthetic process and structural characterizations of the layered VS_2 nanosheets. **a** Schematic illustration of hydrothermal synthesis of layered VS_2 nanosheets. **b** XRD pattern, **c** Raman spectra, **d** SEM image, and **e** SEM-EDX analysis of the as-prepared VS_2 nanosheets

value of 4.0, at room temperature) were adopted as the standard conditions for subsequent studies.

Stability and reusability of VS_2 catalysts

Normally, it is necessary to eliminate the possibility that the catalytic activity is induced by possible leaching ions from VS_2 such as vanadate. As showed in Fig. S3, the leaching solution of 100 μg·mL^{-1} VS_2 exhibits almost no activity compared to that of 100 μg·mL^{-1} VS_2. It demonstrates that the catalytic activity is derived from VS_2 nanosheets themselves rather than the leaching ions. In addition, to test the stability of the catalyst, the XRD pattern and Raman spectra of the catalyst after catalysis (Fig. 1b, c) were also obtained at first. No

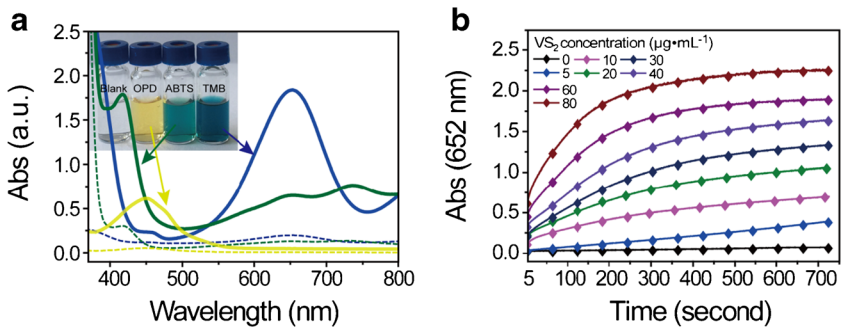


Fig. 2 POx-like catalytic properties of VS_2 nanosheets. **a** UV-vis absorption spectra recording the oxidation of 1 mM ABTS (green line), TMB (blue line), and OPD (yellow line) catalyzed by VS_2 nanosheets (25 μg·mL^{-1}) in the presence of H_2O_2 (20 mM). The corresponding dashed line indicates the incubation of VS_2 with ABTS, TMB, or OPD only. Reaction time $t = 10$ min. **b** Time- and catalyst concentration-dependent absorbance at 652 nm measured from the reaction solutions containing 20 mM H_2O_2, 0.5 mM TMB, and VS_2 nanosheets of different concentrations in 0.1 M acetate buffer (pH 4.0) at room temperature

differences can be seen in the XRD and Raman data when comparing VS_2 nanosheets before and after catalysis. This indicates that the catalyst is still present as vanadium disulfide after catalytic reaction. Further, the thermal stability of VS_2 was examined by heating the catalysts at 60, 80, and 100 °C before catalytic reaction. Fig. S4 shows that although the catalyst loses ~25% activity after 1 h heating treatment at 100 °C, the catalyst still maintains 90% activity after treatment at 60 or 80 °C. This indicates an acceptable thermal stability of VS_2 nanozymes for practical uses. After that, we also examined the reusability of VS_2 as peroxidase catalyst (Fig. S5). Results demonstrate that VS_2 is reusable catalyst, which can maintain over 85% of the catalytic activity after even eight repetitive cycles.

Catalytic mechanism of VS_2 as POx mimetics

The catalyst-induced generation of reactive oxygen species from the decomposition of H_2O_2 is often considered to be the key of catalytic activity. To explore the possible mechanism of catalytic behavior of VS_2 nanosheets, the catalytic reaction was further investigated in coexistence of different radical scavengers. Here, sodium azide (NaN_3), superoxide dismutase (SOD), ascorbic acid (AA) and thiourea were chosen as effective scavengers for $^1O_2$ [23], $O_2^{\bullet-}$ [24], active oxygen free radicals (•OH and $O_2^{\bullet-}$) [25], and •OH [26], respectively. As exhibited in Fig. 3, the absorbance is not obviously affected in coexistence of NaN_3 and SOD, while the absorbance is greatly decreased in coexistence of AA and thiourea. It shows that •OH may be the reactive oxygen species produced in the reaction system. Terephthalic acid can form highly fluorescent product (2-hydroxy terephthalic acid) in the presence of •OH [27]. Therefore, by using terephthalic acid as a fluorescent probe, we further examined the presence of •OH in the catalytic system. The fluorescence

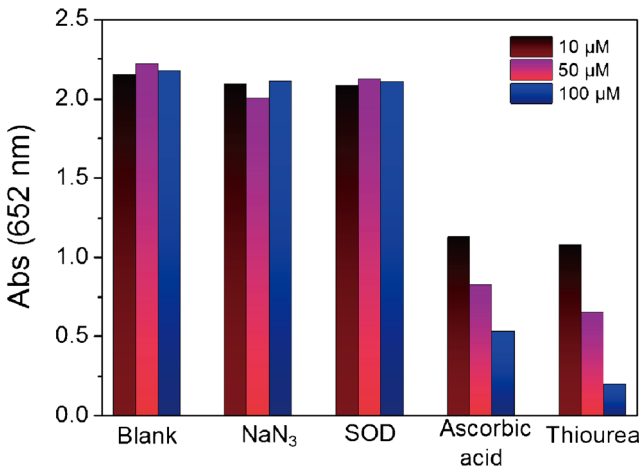


Fig. 3 The catalytic system (50 μg·mL^{-1} VS_2, 20 mM H_2O_2, and 1 mM TMB) responding to different radical scavengers. Condition: 0.1 M acetate buffer (pH 4.0) at room temperature with a reaction time = 15 min

emission spectra in Fig. S6 shows that the fluorescence intensity at 425 nm grows with the increase of the VS_2 sheets. The above reveals that •OH generates from the VS_2-catalyzed decomposition of H_2O_2 in the reaction system, leading to the oxidation of colorimetric substrate. Therefore, the catalytic mechanism of VS_2 peroxidase mimics can be put as follows (Take TMB oxidation as an example):

$$H_2O_2 \xrightarrow{VS_2} \bullet OH \quad (2)$$

$$TMB(colorless) + \bullet OH \xrightarrow{VS_2} oxTMB(blue) + H_2O \quad (3)$$

Further, the apparent steady-state kinetic parameters were determined for the reaction between TMB and H_2O_2. By plotting the initial reaction velocities against substrate concentrations, typical Michealis-Menten curves were observed for both TMB (Fig. 4a) and H_2O_2 (Fig. 4b). The curves were then fitted to the Lineweaver Burk plots (Fig. 4c, d), from which the kinetic parameters, Michaelis-Menten constants (K_m) and maximum initial reaction rates (V_{max}), were calculated.

Generally, a lower K_m value represents a higher affinity between the enzyme and the substrate. As showed in Table 1, VS_2 nanosheets exhibited lower K_m values (0.28 and 3.49 mM) than that of HRP (0.434 and 3.702 mM) [28], indicating higher affinity to both TMB and H_2O_2. It may originate from the large surface active area and the more reactive sites exposed on the surface of VS_2 nanosheets compared to HRP, which has only one iron ion at the active center. In comparison with other POx-like nanozymes in Table 1, VS_2 nanosheets also have comparable affinity for both TMB and H_2O_2. Among them, VS_2 catalysts show smaller K_m value for TMB than that of their TMDs analogues, such as MoS_2 and WS_2. Moreover, VS_2 exhibit remarkable advantages given by V_{max} over most of the nanozymes in Table 1. Therefore, it is reasonable to claim that VS_2 nanosheets exert a superior peroxidase mimicking activity, rivaling natural protein enzyme HRP and many other POx mimetics.

Colorimetric detection assay for H_2O_2 and glucose

On the basis of the robust peroxidase property of the V_2S catalysts, we designed a colorimetric assay for determination of glucose by utilizing the catalyzed TMB–H_2O_2 colored reaction coupled with glucose oxidase (GOx) (Scheme 1). Briefly, GOx was first incubated with glucose in optimal condition to catalyze the oxidation of glucose to produce gluconic acid and hydrogen peroxide in the presence of oxygen. Subsequently, the formed hydrogen peroxide was utilized to switch on the reaction between the VS_2 catalysts and TMB, resulting in the generation of blue color oxidation product. Eventually, the formed color and absorbance was employed as colorimetric signal indirectly probing the glucose content.

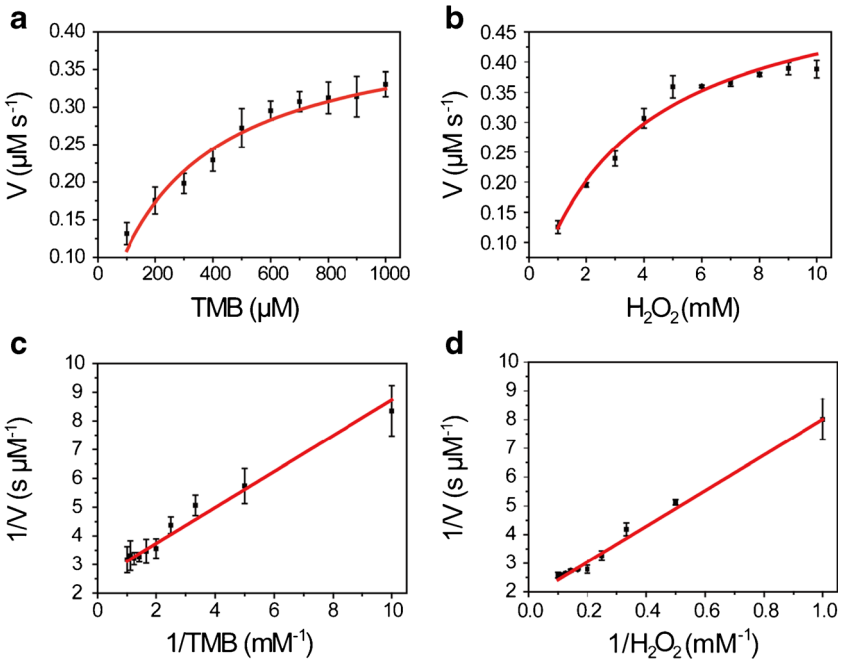
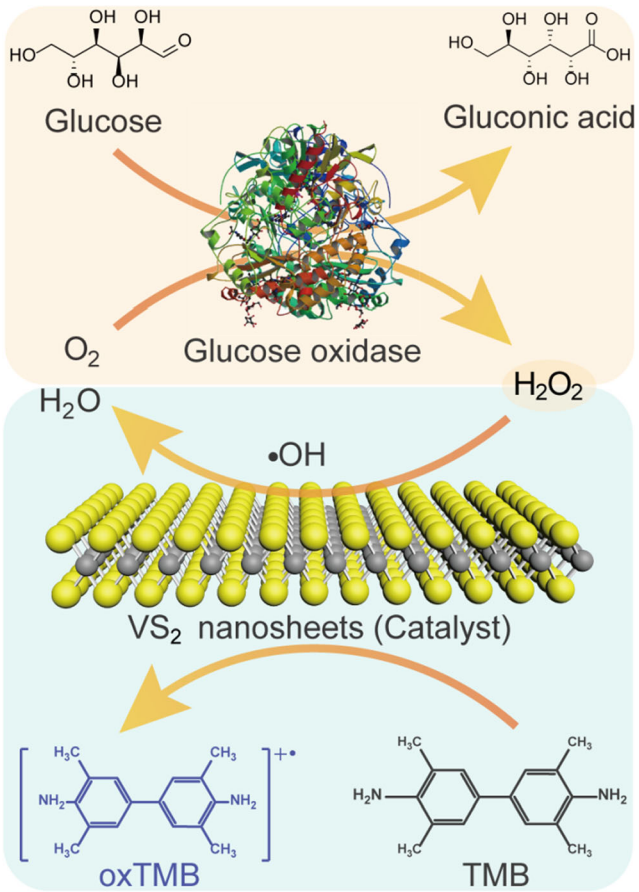


Fig. 4 The steady-state kinetic analysis of VS_2 nanosheets. **a** 10 mM H_2O_2 with different concentrations of TMB. **b** 0.5 mM TMB with different concentrations of H_2O_2. **c**, **d** Double-reciprocal plots of (A, B), respectively. Condition: 50 μg·mL^{-1} VS_2 in acetate buffer (pH 4.0) at room temperature

To verify the feasibility of this principle, hydrogen peroxide was assigned as detection target directly for the detection assay at first. Figure 5a shows a characteristic absorbance (652 nm) versus H_2O_2 concentration response plot of the H_2O_2 assay. The response is linear ($R^2 = 0.999$) in the H_2O_2 concentration range from 2 to 100 μM with a detection limit (LOD, 3σ) of 0.57 μM (Fig. 5b). This result indicates the VS_2-H_2O_2-TMB reaction system is dependent on H_2O_2 concentration and suitable for detection assay. Subsequently, glucose detection assay was carried out using VS_2-H_2O_2-TMB reaction combined with glucose-GOx system. Then, the UV-vis absorption intensity at 652 nm was monitored as a function of the glucose concentration. As shown in Fig. 5c, the glucose detection curve is observed to be linear ($R^2 = 0.996$) between

Table 1 Comparison of Michaelis-Menten constants (K_m) and maximum initial reaction rates (V_{max}) of the oxidation reaction catalyzed by VS_2 nanosheets, HRP, and some peroxidase mimetics

Catalysts	K_m(mM)		V_{max} (10^{-8} M•s^{-1})		Reference
	TMB	H_2O_2	TMB	H_2O_2	
VS_2 NSs	0.28	3.49	41.6	55.7	This work
HRP	0.434	3.702	10	8.71	[28]
GO-COOH	0.0237	3.99	3.45	3.85	[6]
WS_2 NSs	1.83	0.24	4.31	4.52	[29]
MoS_2 NSs	0.525	0.0116	5.16	4.29	[30]
CuZnFeS NCs	2.2	0.07	39	0.56	[27]
Fe_3S_4	0.160	1.158	1.146	2.168	[31]
$Cu(OH)_2$ SCs	2.448	0.199	44.8	42.5	[32]
$Cu_2(OH)_3Cl$-CeO_2	12.36	11.61	10.62	8.15	[33]
$Fe_2(MoO_4)_3$-F	1.126	0.105	3.73	7.51	[34]
β-CD-Cu-NCs	0.543	29.16	43.4	45.2	[35]



Scheme 1 Schematic diagram of vanadium disulfide-based POx-mimicking catalysis and glucose detection

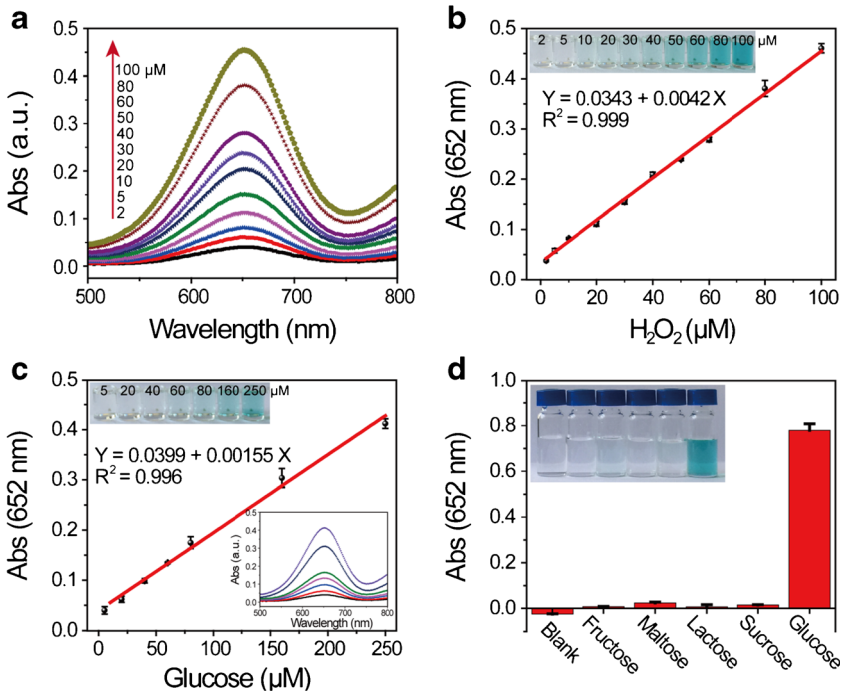


Fig. 5 H_2O_2 and glucose detection assays using VS_2 POx mimetics. **a** H_2O_2 concentration dependent UV-vis absorption change. **b** H_2O_2 and (**c**) glucose assay calibration curves obtained from UV-vis spectra measurement. The inset photograph shows the visually recognizable color change of the reaction system, accordingly. Condition: 50 μg·mL^{-1} VS_2 and 1 mM TMB, 10 min incubation in acetate buffer (pH 4.0) at room temperature. **d** Selectivity test of glucose over other coexisting substances in fruit juice. The photograph shows the corresponding visual color change. Condition: 1.5 mM glucose and 5 mM other coexisting substances

5 and 250 μM with the LOD estimated to be 1.54 μM. Moreover, the VS_2 nanosheet-based assay can compete with, or even surpass certain other nanozyme-based glucose assays (see Table S1). The selectivity of the VS_2 catalyst for glucose detection was further examined, by monitoring the absorbance change upon addition of various glucose analogues. No significant interference can be observed from fructose, maltose, lactose, or sucrose (Fig. 5d), confirming our colorimetric assay exhibits a good selectivity for glucose. Using this glucose assay, we can detect glucose in food samples such as fruit juices. As shown in Table S2, the results obtained by our colorimetric assay agree with the results obtained by a commercial enzymatic Glucose (HK) Assay Kit (Sigma Aldrich). It validates the reliable colorimetric method for glucose determination in food samples. The good performance of such colorimetric glucose assay based on VS_2 nanosheets stimulates promising biocatalytic and analytical applications of VS_2 in the future.

Conclusion

In conclusion, we have hydrothermally synthesized layered VS_2 nanosheets, which are discovered to act as peroxidase-like catalysts to oxidize the peroxidase substrates in the aid of H_2O_2. Seeing from our results, the VS_2 catalysts can be readily available substitute for HRP and many other peroxidase-like nanozymes. It is verified that VS_2 nanosheets catalyze the decomposition of H_2O_2 in acidic pH into •OH, which results in the oxidation of a substrate to form colored reaction product. Relying on this discovery, a colorimetric assay for glucose determination in fruit juice was successfully developed, with high selectivity and low LOD (1.54 μM). In this study, two- dimensional VS_2 is demonstrated to be a promising building block for analytical and biological systems. However, to achieve advanced applications, future investigations are still needed in promoting the chemical stability and biocompatibility of VS_2. Our work lays the foundation for imitating natural biocatalysis via VS_2 nanosheets, facilitating the rise of novel applications in biotechnology and analytical chemistry.

Acknowledgements This research was financed by Grants from National Natural Science Foundation of China (No. 21675127), Fundamental Research Funds for the Northwest A&F University of China (2014YB093, 2452015257), and Development Project of Qinghai Key Laboratory (No. 2017-ZJ-Y10).

Compliance with ethical standards The author(s) declare that they have no competing interests.

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