

5,5-二乙烯基-2,2-联吡啶:可能的新型锌离子荧光探针

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摘要:生物体系里微量元素锌在发育、新陈代谢和疾病的发生等多个领域扮演了重要的角色,定性和定量测量锌的含量将帮助我们理解锌的生物学意义。我们偶然发现 5,5-二乙烯基-2,2-联吡啶(DVBP)在二氧六环里对锌离子呈现出类似比例计量型荧光探针的特性;即有 2 个荧光发射峰而且长波长峰随锌离子的增加按比例增长。但 DVBP 难溶于水而且 2 个荧光发射峰距离太近从而相互干扰,我们尝试利用硅氢反应将 DVBP 固定在多孔硅上制备感应锌离子的光极,我们发现多孔硅固相载体上的联吡啶通过与锌离子的螯合后荧光增强约 8 倍,比 DVBP 在溶液里与锌离子螯合后的荧光增强(约 4.5 倍)还要多,联吡啶对锌离子的荧光响应是一个值得继续探索的领域。

关键词: 联吡啶; 锌离子; 荧光探针; 多孔硅

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5,5-Divinyl-2,2-bipyridine: a Possible New Prototype of Fluorescent Probe for Sensing Zn²⁺

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Abstract: In biological systems, zinc-ion plays an important role in growth, metabolism, disease occurrence and other aspects. Qualitative and quantitative measurements of zinc-ion will help us understand the biological significance of zinc-ion. We incidentally found that 5, 5-divinyl-2, 2-bipyridine(DVBP) in 1, 4-dioxane presented a ratiometric sensing feature toward Zn²⁺, which possesses dual fluorescent emissions and the intensity of the long-wavelength emission increases ratiometricly with increasing the concentration of Zn²⁺. However the non-solubility of DVBP in aqueous media and its limitation for practical applications with the two emission bands overlapped severely render us to search for alternative sensing approaches. We immobilized DVBP on porous silicon(PS) supporting matrices for fabrication of Zn²⁺-sensing optode. We found that Zn²⁺ even enhanced the bipyridyl fluorescence ~8 times, stronger than its enhancement of DVBP's fluorescence(~4.5 times) in solution. Further research on fluorescent response of bipyridine derivatives to Zn²⁺ should be continued.

Key words: bipyridine; zinc ion; fluorescent probe; porous silicon

Fluorescence probes serve as useful tools in biological investigations^[1]. Zinc ion(Zn²⁺) is one of the targets in the design of ratiometric probes, because this

metal ion is of great interest in the field of neurobiology. Fluorescent indicators that allow real-time imaging of Zn²⁺ in living cells are invaluable tools for understand-

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ding Zn^{2+} homeostasis^[2]. Recently, the zinc fluorescent probes have been developed by leaps and bounds, and some new fluorescent probes were invented and discovered, including small chemical molecular probes and large biological probes^[3]. The most attractive Zn^{2+} sensors are ratiometric fluorescent sensors due to their ability to quantify Zn^{2+} in complicated bio-systems. A feature of the ratiometric probe is the ratio change of dual fluorescent emissions upon chelation with Zn^{2+} . The metal chelating enhanced fluorescence is the base for sensing metal elements such as Zn^{2+} . Incidentally we found that 5, 5-divinyl-2, 2-bipyridine (DVBP) in 1, 4-dioxane presented a similar feature of the ratiometric fluorescent probe toward Zn^{2+} . It is the first time to report the ratiometric fluorescent feature of 2, 2-bipyridine derivatives, as we know. However the two emission bands of DVBP are overlapped severely and the ratios cannot be determined certainly. Further DVBP is not soluble in aqueous media and thus it cannot be practically used in aqueous media for sensing Zn^{2+} .

An alternative approach for practical application is the immobilization of fluorescent probes on supporting matrices for optode fabrication. Immobilization is probably the most important step to govern the lifetime of the optode sensor. Poor immobilization results in leaching and consequently worse sensing. Several approaches are used for immobilization: for example, physical entrapment, electrostatic association, and covalent immobilization^[4]. Covalent immobilization is the most efficient technique for preparation of optode sensors because it prevents the leach of the dye from the supporting matrices, which results in a long sensor lifetime. However, there are only a very few articles reporting zinc sensing optodes based on covalently immobilized probes^[5]. Such a zinc sensing approach is an attractive challenge for scientists. Our experience on porous silicon(PS) based sensing^[6] renders the immobilization of DVBP on PS for sensing zinc feasible. Due to its trivial synthesis and unique optical properties^[7], PS has potential applications such as fluorescent tags for biological imaging with brighter and much more stable fluorescence to photo-bleaching than organic dyes^[8-9].

Combination of PS and fluorescent probes might open a new view to scientists in zinc-sensing. In this report, we further immobilized DVBP on to PS and measured its fluorescence before and after exposure to Zn^{2+} . The DVBP-grafted PS supporting matrices bring in the following advantages: stabilization of the nanostructures of PS and greater fluorescence enhancement toward Zn^{2+} than in solution. This initial work might open a completely new window to sensing Zn^{2+} qualitatively and quantitatively.

1 Experimental

1.1 Chemicals and reagents

Silicon wafers were purchased from Huajing Microelectronics (China). 5, 5-Divinyl-2, 2-bipyridine was synthesized in our lab according to a reference^[10]. Other reagents such as $\text{Ni}(\text{OAc})_2$, tetrahydrofuran (THF) and 1, 4-dioxane are all in analytical grade. Single side polished and $\langle 100 \rangle$ oriented p-type silicon wafers($5\sim 8\ \Omega\cdot\text{cm}$ resistivity) were boiled in 3:1 (V/V) concentrated $\text{H}_2\text{SO}_4/30\%\ \text{H}_2\text{O}_2$ for 30 min and then rinsed copiously with MILLI Q water(resistance $> 18\ \text{M}\Omega\cdot\text{cm}$) and pure ethanol.

1.2 Fluorescence spectra

Fluorescence spectra of DVBP (5 mmol) in 3 mL 1,4-dioxane titrated by Zn^{2+} ($10\ \text{mmol}\cdot\text{L}^{-1}\ \text{ZnAc}_2$ in water and $10\ \mu\text{L}$ per addition) were recorded with an AMINCO Bowman Series 2 spectrometer at room temperature. While those of PS samples were measured by a SLM 4800 DSCF/AB2 fluorescence spectrometer using an exciting Xe laser at 366 nm in air.

1.3 Organic modification of PS

The hydrogen-terminated silicon surfaces (Si-H) were made by electrochemical etch in a 1:3 (V/V) pure ethanol and 40% aqueous HF for 8 min at a current density of $60\ \text{mA}\cdot\text{cm}^{-2}$. After etching, samples were rinsed with pure ethanol and dried under a stream of dry nitrogen prior to use.

DVBP was grafted onto PS in 4 mL dry 1,4-dioxane solution containing $44.8\ \text{mmol}\cdot\text{L}^{-1}$ DVBP under microwave irradiation at $300\ \text{W}\cdot\text{min}^{-1}$ for 10 min. Excess un-reacted and physico-adsorbed reagents were removed by rinsing three times with toluene and by

sonication in toluene for a few minutes and dried. Then, the chip was exposed to Zn^{2+} , and washed with copious MILLI Q water and dried with nitrogen before observation by fluorescence microscope.

1.4 FTIR measurements

Transmission infrared Fourier-transform (FTIR) spectra were recorded with a Bruker v80 spectrometer at 4 cm^{-1} resolution. Typically 32 scans were acquired per spectrum. The samples were mounted in a vacuum chamber. A planar Si <100> wafer was used as the background reference. The spectra were recorded in absorbance mode.

2 Results and discussion

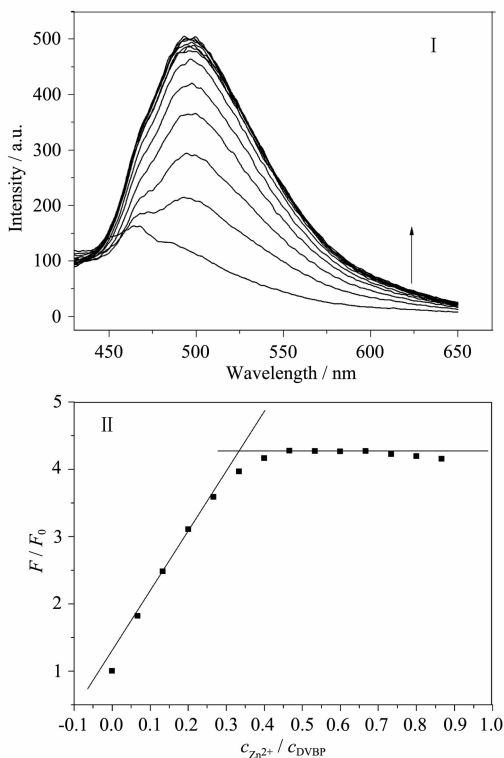
2.1 Fluorescent titration of DVBP by zinc ions

DVBP is an unexceptionable conjugated system, and its fluorescence is very weak in non-polar solutions. Incidentally we found that DVBP in 1, 4-dioxane (Fig.1-I) had dual fluorescence emissions at 460 and 488 nm and zinc ions enhanced the latter emission greatly,

which is similar to the feature of fluorescent ratiometric probes. Zn^{2+} as the second most abundant transition metal ion in human body plays essential roles in cellular proliferation, signal transduction and gene transcription and so on. Zinc metabolism disorder has been correlated to many neurodegenerative diseases. Sensing Zn^{2+} by fluorescent dyes quantitatively is a hot research topic presently. The fluorescent enhancement of DVBP by Zn^{2+} is due to the chelation of nitrogen atoms with $3d^{10}$ of Zn^{2+} . It is the stoichiometric chelation between Zn^{2+} and ligands that promises excellent fluorescent quantification of Zn^{2+} . In addition, Zn^{2+} has a stable chemical valence and rarely goes with redox reactions in biological environment, which guarantees the stoichiometric chelation. Fig.1-I shows the spectral evolution and Fig.1-II the titration curve of DVBP by Zn^{2+} in 1, 4-dioxane. One fluorescent emission originally at 488 nm is enhanced greatly after addition of Zn^{2+} and it also red-shifts to 500 nm, while the other emission at 460 nm as the shoulder of the 500 nm band is brought up too but we cannot make a clear judgment whether it is enhanced by Zn^{2+} .

Fig.1-II exhibits the titration curve presented by F/F_0 (F is the fluorescence intensity of the sample during titration and F_0 the fluorescence intensity of DVBP in 1, 4-dioxane without Zn^{2+}) against the mole ratio $\text{Zn}^{2+}/\text{DVBP}$ at 500 nm. It is easy to see that the fluorescence intensity increases linearly with increasing Zn^{2+} before the molar ratio reaches ~ 0.35 , and then stays nearly the same. The molar ratio 0.35 indicates that the stoichiometric coordination is 3 DVBP molecules (or six N atoms) against one Zn^{2+} . When the fluorescent intensity of DVBP reaches its maximum value, increasing Zn^{2+} concentration has little effect on the intensity any more and it remains nearly the same.

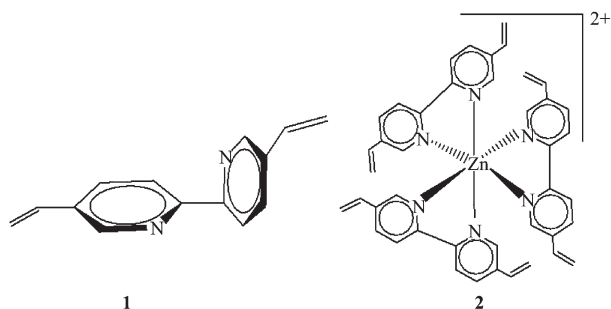
An excellent fluorescent ratiometric probe should present two individual emission bands without overlapping. With the addition of Zn^{2+} , one will increase and the other will decrease. The molecular mechanism is generally explained as tautomerization of the ligand dye. One mesomer of the dye converts to the other mesomer structure with the addition of Zn^{2+} due to chelation. The latter mesomer dominates the system after



The arrow in (I) indicates the spectral titration sequences by sequential addition of $10\text{ }\mu\text{L}$ of $10\text{ mmol}\cdot\text{L}^{-1}\text{ ZnAc}_2$ into 3 mL 1, 4-dioxane containing 5 mmol DVBP

Fig.1 Fluorescent spectral evolution (I) and titration curve (II) of DVBP upon addition of Zn^{2+}

chelation, and therefore its fluorescence intensity increases. In our case, DVBP does not possess such a molecular structure for tautomerization. We suggest that the two emission bands be due to conformational isomerism. One stereoisomer is the individual DVBP molecule (**1** in Scheme 1) in 1, 4-dioxane which possesses a tilt angle between two pyridyl rings. The other stereoisomer is the coordination compound of DVBP with Zn^{2+} . Chelating Zn^{2+} with N of DVBP affects the lone electron pair of the N atom which interacts with those from the pyridyl group. Due to the coordination effect, two pyridyl rings are regulated by Zn^{2+} to the same plane (**2** in Scheme 1). Coordination with Zn^{2+} increases the conjugation degree of DVBP over the whole molecule and thus results in red-shift and enhancement of the fluorescent emission at 500 nm. The tilted DVBP stereoisomer (**1**) does not have a perfect conjugation over the whole molecule and so its fluorescence is weak and is at the short wavelength of 460 nm.



1: a stereoisomer with a tilted angle between two pyridyl rings; **2:** the other stereoisomer of DVBP with two pyridyl rings in the same plane after coordination with Zn^{2+}

Scheme 1 Two stereoisomer structures of DVBP

Although we found the ratiometric feature of DVBP by coordination with Zn^{2+} in 1, 4-dioxane, DVBP is not soluble in water and therefore practically it cannot be used in the biological system in its present form. From the practical point of view, immobilization of DVBP in solid supports might be an alternative approach for sensing Zn^{2+} . For this purpose, we further investigated the DVBP-grafted PS for fluorescent measurements before and after exposure to Zn^{2+} in next sections.

2.2 FTIR spectra of PS and of DVBP-modified PS

The grafting of DVBP on PS was confirmed by

transmission FTIR in Fig.2. The spectrum of the hydrogen-terminated PS (Fig.1-PS) exhibits a typical tripartite band for SiH_x ($x=1\sim3$) stretching modes ($2\,087\text{ cm}^{-1}$ for SiH , $2\,114\text{ cm}^{-1}$ for $\nu\text{ SiH}_2$ and $2\,138\text{ cm}^{-1}$ for $\nu\text{ SiH}_3$). The SiH_x bending modes are observed at 916 , 669 and 630 cm^{-1} . After grafting DVBP (Fig.1-DVBP), besides a strong Si-O band at $\sim 1\,200\text{ cm}^{-1}$ and the un-reacted SiH_x bands, two alkyl CH_2 bands at $2\,927$ and $2\,857\text{ cm}^{-1}$ are respectively assigned to CH_2 asymmetric and symmetric stretching modes, and multiple bands of $1\,630$, $1\,598$, and $1\,549\text{ cm}^{-1}$ respectively to skeletal stretching modes of pyridine. All bands of SiH_x after modification are still observable but exhibit a significant decrease, meaning the remaining residues of SiH_x species.

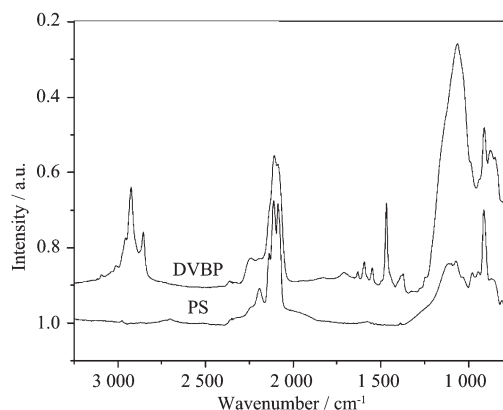


Fig.2 Transmission FTIR of PS and DVBP-modified PS

2.3 Fluorescence of DVBP-modified PS samples

The fluorescent spectra of PS, DVBP-grafted PS, and DVBP-grafted PS exposed to Zn^{2+} are shown in Fig.3. It is well known that PS is fluorescent and its e-

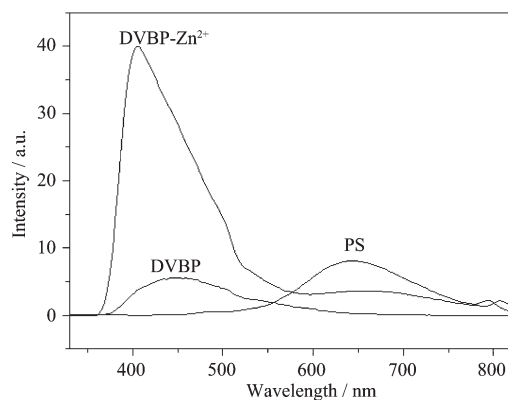


Fig.3 Photoluminescence of modified PS samples: PS, porous silicon; DVBP, DVBP modified PS; DVBP-Zn, DVBP modified PS exposed to Zn^{2+} ($100\text{ mmol}\cdot\text{L}^{-1}$ in aqueous solution)

mission wavelength is located at about 630 nm. After grafting DVBP, we found that the fluorescent emission from the modified PS surface was at around 450 nm, which was contributed by 2,2-bipyridine. The fluorescent emission at about 630 nm from the nano-structured PS disappears completely after modification and washing steps, which is in accordance to our previous report^[6]. After exposing the chip to $0.1 \text{ mol} \cdot \text{L}^{-1} \text{ Zn}^{2+}$, the fluorescence from the chip surface slightly blue-shifted to 405 nm and its intensity was enhanced 8 times. Comparing the fluorescence spectra on the PS supporting matrices in Fig.3 from those in 1, 4-dioxane in Fig.1-I, we found that coordination of Zn^{2+} with the bipyridine ligand in supporting matrices relatively enhanced the fluorescence greatly (8 times versus 4.5 times). While on PS there is only one fluorescence band and its position is at a shorter wavelength of 405 nm. This is due to the grafting reaction of both end vinyl groups of DVBP with SiH_x species to form Si-C bonds. The loss of two end double bonds causes the decrease of the whole π conjugation degree, which results in a fluorescent blue-shift. By means of the fluorescent enhancement on the supporting matrices of PS, we could establish a quantitative analysis method to analyze Zn^{2+} in the future.

3 Conclusion

To summarize, we first reported the ratiometric sensing feature of 2,2-bipyridine derivatives toward Zn^{2+} in 1, 4-dioxane. We explained the dual emissions by conformational isomerism. Our report opened a small window for a new prototype of Zn^{2+} -sensing fluorescent probes of 2, 2-bipyridine derivatives. The present work has limitations: the two emission bands of DVBP are overlapped severely and DVBP is not soluble in aqueous media and thus DVBP cannot be practically used in aqueous media for sensing Zn^{2+} . However we tried the supporting matrices based sensing and surprisingly found that Zn^{2+} even enhanced 2, 2-bipyridines fluorescence (~8 times) stronger than in solution (~4.5 times).

Immobilization of fluorescent probes on supporting matrices renders selection of dye molecules wider since we do not need to modify the dye molecule to a water-soluble form. More work on the fluorescent response of bipyridyl derivatives to Zn^{2+} will be followed.

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