Exploration and application of a highly sensitive bis(salamo)-based fluorescent sensor for $\text{B}_4\text{O}_7^{2−}$ in water-containing systems and living cells

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A highly selective fluorescent sensor $\text{H}_4\text{L}$ based on a bis(salamo)-type compound with two $\text{N}_2\text{O}_2$ chelating moieties as ionophore was successfully developed. Sensor $\text{H}_4\text{L}$ was found to have excellent selectivity for $\text{B}_4\text{O}_7^{2−}$ over many other anions ($\text{Br}^{−}$, $\text{Cl}^{−}$, $\text{CN}^{−}$, $\text{CO}_3^{2−}$, $\text{HCO}_3^{−}$, $\text{H}_2\text{PO}_4^{−}$, $\text{HSO}_4^{−}$, $\text{NO}_3^{−}$, $\text{OAc}^{−}$, $\text{S}_2\text{O}_3^{2−}$, $\text{SCN}^{−}$, $\text{SO}_4^{2−}$, Hcy (homocysteine) and $\text{H}_2\text{O}_2$), and it exhibited an approximately 150-fold enhancement of the fluorescence response to $\text{B}_4\text{O}_7^{2−}$ in Tris-HCl buffer (DMF/H$_2$O = 9:1, v/v, pH = 7) solutions. Significantly, its fluorescence intensity was enhanced in a linear fashion with increasing concentrations of $\text{B}_4\text{O}_7^{2−}$. The detection limit of sensor $\text{H}_4\text{L}$ towards $\text{B}_4\text{O}_7^{2−}$ was $8.61 \times 10^{-7}$ M. The test strips could conveniently, efficiently and simply detect $\text{B}_4\text{O}_7^{2−}$ ions in Tris-HCl buffer (DMF/H$_2$O = 9:1, v/v, pH = 7) solutions. Furthermore, sensor $\text{H}_4\text{L}$ showed excellent membrane permeability in living cells, and it was successfully used to monitor intracellular $\text{B}_4\text{O}_7^{2−}$ by confocal luminescence imaging.

Metal ions and anions both play a key role in daily life1–4. Borate, an essential element in the earth, is widely used in industry, agriculture and medicine. For example, borate has widespread use in a solid lubricant in industry, and it may also be applied in welding repair to refrigeration equipment. In medicine, borate could be used for the anti-corrosion of the skin and mucous membranes as well as in the treatment of cancer. In animal medicine, as a feed additive, the research on borate has been attracting increasing attention. Nevertheless, abusing borates not only damages the environment but also endangers human health. Hence, the development of a rapid and convenient detection method for $\text{B}_4\text{O}_7^{2−}$ could be of interest.

Up until now, with the development of optical sensors for recognizing heavy and transition metal ions in living organisms5–15, intense efforts have been devoted to the design and synthesis of high sensitivity fluorescent sensors due to their low cost and rapid response as well as the easy operability of the fluorescent technique16–22. According to the relevant literature, the metal complexes of $\text{N}_2\text{O}_2$ salen-type ligands and corresponding analogues could be used in catalysis23,24, nonlinear optical materials and magnetic materials25–34, supramolecular architecture35,36, ion recognition37–45, biological fields and so forth46–52. Today, studies on the participation of salamo-type compounds in ion recognition have yet to be explored53–63. Notably, compared with most of the known fluorescent probes for $\text{Zn}^{2+}$, $\text{Cu}^{2+}$, and $\text{CN}^{−}$, there are relatively few reports on fluorescent probes for $\text{B}_4\text{O}_7^{2−}$.

Herein, we have designed and synthesized a bis(salamo)-type sensor $\text{H}_4\text{L}$ for the recognition of $\text{B}_4\text{O}_7^{2−}$ in Tris-HCl buffer (DMF/H$_2$O = 9:1, v/v, pH = 7) solutions. The UV–vis absorption spectra and fluorescence titration experiments for sensor $\text{H}_4\text{L}$ were investigated and the results indicated that sensor $\text{H}_4\text{L}$ has a high selectivity for $\text{B}_4\text{O}_7^{2−}$ over many other ions based on the change in color visible to the naked eye and the fluorescence intensity at a low concentration as well as a mild environment.

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Results and Discussion

The selectivity of sensor H4L to B4O7^{2−}. A series of host-guest recognition experiments were carried out to investigate the B4O7^{2−} recognition ability of sensor H4L with various anions and some compounds, B4O7^{2−}, Br^−, Cl^−, CN^−, CO3^2−, HCO3^−, H2PO4^−, HSO4^−, NO3^−, OAc^−, S2O3^2−, SCN^−, SO4^{2−}, Hcy and H2O2 in Tris-HCl buffer (DMF/H2O = 9:1, v/v, pH 7) solutions. As shown in Fig. S2a, all of the examined anions show the same absorption peaks with sensor H4L, however, only the addition of B4O7^{2−} displayed the highest absorbance under the same reaction conditions. There are no isosbestic points due to the differences in binding abilities between sensor H4L and all of these anions.

The interaction of sensor H4L and B4O7^{2−} was evaluated by a UV–vis titration method. As shown in Fig. S2b, with increasing concentrations of B4O7^{2−} (0.001 M) from 0.0–39.0 equiv. in Tris-HCl buffer (DMF/H2O = 9:1, v/v, pH 7) solutions, the absorbance showed a linear increase when the ratio of [B4O7^{2−}]/[H4L] is below 39:1, and the absorbance no longer changes when the ratio reached 39:1.

Effect of the pH on sensor H4L. In order to remove the interference by protons during the detection of B4O7^{2−} and to find the optimal sensing conditions, further tested was performed in the pH range of 1 to 12. As shown in Fig. 1, the results obtained show no dramatic spectral changes of sensor H4L in the wide pH range of 1–12, suggesting that sensor H4L was very stable. The H4L-B4O7^{2−} displayed a strong fluorescence intensity in the pH range of 1–7. The results above clearly indicate that sensor H4L can be employed as a sensitive relay-sensor to recognize and distinguish B4O7^{2−} in a wide pH range.

Fluorescence detection of sensor H4L towards B4O7^{2−}. Selectivity is a very important parameter to evaluate the performance of a fluorescence chemosensor. The fluorescence emission spectral responses of sensor H4L to various anions and some compounds (B4O7^{2−}, Br^−, Cl^−, CN^−, CO3^2−, HCO3^−, H2PO4^−, HSO4^−, NO3^−, OAc^−, S2O3^2−, SCN^−, SO4^{2−}, Hcy and H2O2) were evaluated in Tris-HCl buffer (DMF/H2O = 9:1, v/v, pH 7) solutions. As shown in Fig. 2a, all of the examined anions did not display any obvious response to sensor H4L, and
Figure 3. Fluorescence emission spectra of sensor H₄L (0.01 mM) upon the subsequent addition of B₄O₇²⁻ (0–39 equiv. λₑx = 323 nm) in Tris·HCl buffer (DMF/H₂O = 9:1, v/v; pH = 7) solutions.

only after the addition of B₄O₇²⁻ did, sensor H₄L produce a significant enhancement of the fluorescence intensity at 430 nm (λₑx = 323 nm). These results suggested that sensor H₄L displayed an excellent selectivity for B₄O₇²⁻ over all of the other anions tested.

To further explore the high selectivity of sensor H₄L for B₄O₇²⁻ in practice, we also investigated the ability of sensor H₄L to detect B₄O₇²⁻ in the presence of equivalent and excess amounts of other anions, to determine whether they would interfere with coordination between sensor H₄L and B₄O₇²⁻. As shown in Fig. 2b, when anions and some compounds, including Br⁻, Cl⁻, CN⁻, CO₃²⁻, HCO₃⁻, H₂PO₄⁻, HSO₄⁻, NO₃⁻, OAc⁻, S₂O₃²⁻, SCN⁻ and SO₄²⁻, Hcy and H₂O₂, were separately added into a mixed solution of sensor H₄L and B₄O₇²⁻, the fluorescence intensity had little or negligible change. Hence, fluorescence interference experiments of various anions revealed that other anions could not affect the sensing process of sensor H₄L for B₄O₇²⁻. In order to further understand the binding behavior of H₄L with B₄O₇²⁻, the ¹H NMR spectra experiments of H₄L and H₄L-B₄O₇²⁻ were also performed in DMSO-d₆. The phenolic O-H in H₄L has completely disappeared upon the addition of B₄O₇²⁻, and all protons of the aromatic ring and aldimine CH=N in H₄L were shifted down-field (Fig. S3). These changes may be due to the destruction of intermolecular electrostatic and hydrogen-bond interactions after the addition of B₄O₇²⁻ to H₄L.

The fluorescence enhancement of the sensor H₄L response to B₄O₇²⁻ may be attributed to that borates are hydrolyzed to form boric acid:

\[
[B_4O_6(OH)_4]^{2-} + 5H_2O \rightarrow 4H_3BO_3 + 2OH^- \rightarrow 2H_2BO_3 + 2B(OH)_4^-
\]

Four coordinated organoboron compounds based on N,O-chelation are constructed mainly by structures 1, 2 and 3 as the ligand backbone (Fig. S4a). The weak fluorescence of sensor H₄L was attributed to the lone pairs of electrons on the nitrogen atoms, which lead to intra-molecular photoinduced electron transfer (PET). Due to the lack of electronic properties, the Lewis bases such as the N atoms of the salamo moieties from the H₄L unit coordinate to the B atoms, resulting in a unique electronic structure and optical properties after B atoms are incorporated into the conjugated system. Four coordinated organoboron compounds can produce strong fluorescence with the excitation of light²⁴. On the other hand, sensor H₄L exhibited a very weak fluorescence intensity due to the photoinduced electron transfer process from the hydroxy oxygen atom to amino groups. However, when sensor H₄L was coordinated with a B₄O₇²⁻ ion, the chelation-enhanced fluorescence process would be started, and the photoinduced electron transfer process would be inhibited at the same time (Fig. S4b). Hence, an obvious enhancement of the fluorescence intensity was observed.

Fluorescent titration was carried out to gain more insight into the recognition properties of sensor H₄L as a B₄O₇²⁻ probe. As shown in Fig. 3, without B₄O₇²⁻, sensor H₄L had nearly no fluorescence. However, with increasing concentrations of B₄O₇²⁻, the fluorescence intensity was remarkably increased at 430 nm. Significantly, a good linear relationship between the fluorescence intensity and the B₄O₇²⁻ concentration could be obtained (\(R^2 = 0.95873\)), which is based on the fluorescence titration experiment. It can be seen that the fluorescence intensity change was nearly linear with the increase of concentration of B₄O₇²⁻ (Fig. S5). For many practical applications, it is very meaningful to detect the analytes at low concentrations. Meanwhile, based on the corrected Benesi-Hildebrand formula, the binding constant for the binding of B₄O₇²⁻ to sensor H₄L was calculated as 4.72 × 10⁴ M⁻¹. The detection limit (LOD) could be calculated to be 8.61 × 10⁻⁷ M and the limit of quantitation (LOQ = 2.87 × 10⁻⁶ M) of sensor H₄L for B₄O₇²⁻ anions was also obtained. The LOD and LOQ were calculated based on the following equations:

\[
LOD = 3 \times \delta/S; \quad LOQ = 10 \times \delta/S.
\]
Where $\delta$ ($\delta = 3.9 \times 10^{-5}$) represents the standard deviation of the blank measurements, and $S$ is the slope of the intensity versus sample concentration curve. We investigated the binding stoichiometry and binding affinities of sensor H$_4$L and B$_4$O$_7^{2-}$. A Job's plot analysis for the fluorescence intensity was also measured by keeping the sum of the initial concentrations of sensor H$_4$L and B$_4$O$_7^{2-}$ constant at 10 $\mu$M (Fig. 4). The experiment was performed in Tris-HCl buffer (DMF/H$_2$O = 9:1, v/v, pH = 7) solutions at an excitation wavelengths of 323 nm. The results indicated that the binding stoichiometry between sensor H$_4$L and B$_4$O$_7^{2-}$ is 1:1.

The realization of a quick response to B$_4$O$_7^{2-}$ is very meaningful for sensor H$_4$L in its practical application in portable sensing devices. To facilitate the use of sensor H$_4$L for the detection of B$_4$O$_7^{2-}$, test strips were made by soaking filter papers in a Tris-HCl buffer (DMF/H$_2$O = 9:1, v/v, pH = 7) solution of sensor H$_4$L followed by exposure to air until complete drying. Intriguingly, the obvious fluorescence color changes were observed immediately from gray to light blue in visible light when B$_4$O$_7^{2-}$ anions were added. Therefore, sensor H$_4$L exhibited excellent fluorescence sensing performance, which would be very useful for the fabrication of sensing devices with fast and convenient detection of B$_4$O$_7^{2-}$ (Fig. 5).

In order to be applied in real life and to find the optimal sensing conditions, the fluorescence intensity of sensor H$_4$L over a period of time in the presence of B$_4$O$_7^{2-}$ was determined in Tris-HCl buffer (DMF/H$_2$O = 9:1, v/v, pH = 7) solutions. As shown in Fig. S6a, it was found that there were nearly no changes in the fluorescence intensity of H$_4$L-B$_4$O$_7^{2-}$ over a period of time, suggesting that H$_4$L-B$_4$O$_7^{2-}$ was very stable. Additionally, the fluorescence intensities at different temperatures were also determined. As shown in Fig. S6b, H$_4$L exhibited satisfactory B$_4$O$_7^{2-}$ sensing abilities when the temperature was in the range of 0–90 °C. Therefore, it was demonstrated that sensor H$_4$L could work in a short time and at room temperature, and it can be applied in real life.

Prior to the imaging experiments, the cytotoxicity of H$_4$L at different concentrations (0–100 $\mu$M) was evaluated through 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays in BHK-21 cells. The results after 48 h revealed that H$_4$L exhibited almost no toxicity or low toxicity (Fig. 6). The ability of sensor H$_4$L to detect B$_4$O$_7^{2-}$ in living cells was further studied by confocal luminescence imaging. As seen in Fig. 7, the BHK-21 cells incubated with sensor H$_4$L (30 $\mu$M) alone for 30 min at 37°C maintained a good shape and were viable, the solvent for the H$_4$L concentrate is DMSO, and they also showed very good intracellular fluorescence. Interestingly, an enhanced intracellular fluorescence was detected in cells containing sensor H$_4$L incubated with...
B₄O₇²⁻ for 3 h. From confocal fluorescence images of the BHK-21 cells, it was revealed that sensor H₄L displayed good cell permeability and could be used to detect B₄O₇²⁻ ions in living cells.

In conclusion, we designed and synthesized a bis(salamo)-type sensor H₄L, which showed excellent recognition of B₄O₇²⁻ with different fluorescence changes and changes in color. In addition, the detection limit of the fluorescence response of sensor H₄L to B₄O₇²⁻ is as low as 8.61 × 10⁻⁷ M. This sensing system shows many advantages. The test strips could conveniently, low cytotoxicity, efficiently and simply detect B₄O₇²⁻ in solutions. In addition, the free sensor H₄L was achieved through regeneration by using EDTA and was able to further sense B₄O₇²⁻. We believe that this study provides a potential application for constructing a fluorescent sensor for the highly sensitive and rapidly recognition of B₄O₇²⁻ ions based on different fluorescence intensities and changes in color in practical life.

Figure 6. Cytotoxicity assays of H₄L at different concentrations for BHK-21 cells.

Figure 7. Confocal luminescence images of BHK-21 cells. (a) BHK-21 cells were incubated with sensor H₄L (30 μM) for 30 min at 37 °C and (b) then further incubated with B₄O₇²⁻ (100 μM) for 30 min.
Materials and General Methods

2-Hydroxy-3-methoxybenzaldehyde (99%), methyl trioctyl ammonium chloride (90%), pyridinium chlorochromate (98%) and boron tribromide (99.9%) were purchased from Alfa Aesar. Hydrobromic acid 33 wt% solution in acetic acid was purchased from J&K Scientific Ltd. The other reagents and solvents were analytical grade reagents from the Tianjin Chemical Reagent Factory and were used as received. Melting points were obtained by the use of a microscopic melting point apparatus made by the Beijing Taike Instrument Limited Company and were uncorrected. 1H NMR spectra was determined by a German Bruker AVANCE DRX-400 spectrophotometer. All of the UV–vis and fluorescence spectroscopy experiments were recorded on Shimadzu UV-2550 and Perkin-Elmer LS-55 spectrometers, respectively.

Synthesis of sensor \( H_4L \).

The bis(salamo)-type sensor \( H_4L \) was synthesized according to the previously reported procedure\(^ {70–78} \). The IR, \( ^1 \)H NMR and UV-vis spectra of \( H_4L \) are nearly consistent with the literature data (Fig. S1). The major reaction steps of sensor \( H_4L \) are demonstrated in Fig. 8.

Statistical analysis.

Statistical methods used are detailed at each experiment individually.

Data Availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Author Contributions

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Author Contributions

L.M. Pu, J. Hao and Y.X. Sun performed most of the experiments. X.Y. Li, Y. Zhang and H.T. Long contributed to the writing of the manuscript. W.K. Dong designed the project. X.Y. Li reviewed the manuscript.

Additional Information

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