Simple fluorescence assay for triethylamine based on the palladium catalytic dimerization of benzofuran-2-boronic acid

Akari TANGE, Azumi HIGASHI, Naoya KISHIKAWA and Naotaka KURODA†

Graduate School of Biomedical Sciences, Course of Pharmaceutical Sciences, Nagasaki University, 1-14 Bunkyo-machi, Nagasaki 852-8521, Japan

†To whom correspondence should be addressed

E-mail: n-kuro@nagasaki-u.ac.jp
Abstract

Although benzofuran-2-boronic acid hardly emits fluorescence, it can be rapidly converted to highly fluorescent benzofuran dimer after mixing with palladium catalyst and amine. We found that the fluorescence enhancement accompanying dimerization quantitatively promoted with increasing the concentration of amine. In the present study, we developed a simple fluorescence assay for amines based on the promotive effect. As a result of the fluorescence measurement of the reaction mixture of 19 kinds of typical amines, it was found that tertiary amines including triethylamine (TEA) provided significant fluorescence enhancement. Finally, the fluorogenic reaction could be applied to develop a high-throughput fluorescent microplate assay for TEA with the limit of detection (blank + 3SD) of 0.091 µM.
Introduction

The palladium-catalyzed cross coupling reactions are considered to be efficient to construct carbon-carbon bonds.\textsuperscript{1-3} The Suzuki-Miyaura coupling reaction is a palladium-catalyzed cross-coupling reaction between aryl halides and aryl boronic acids and has been widely used to synthesize various chemicals including pharmaceutics due to the stability of the reagents.\textsuperscript{4-6} In the case of the Suzuki-Miyaura coupling reaction, bases including amines are frequently added to activate aryl boronic acids in order to higher reaction yield.\textsuperscript{7}

On the other hand, we previously reported that benzofuran-2-boronic acid could be used as a fluorescent sensor to detect palladium.\textsuperscript{8} Although benzofuran-2-boronic acid hardly emits fluorescence, the significant fluorescence enhancement can be observed with the addition of palladium in the presence of amine such as triethylamine (TEA). As shown in Fig.1, this phenomenon could be attributed to the conversion of weakly fluorescent benzofuran-2-boronic acid to highly fluorescent benzofuran dimer by the catalytic activity of palladium. Furthermore, in the course of related work, we found that the fluorescence quantitatively increased with increasing the concentration of TEA. Therefore, we considered that the fluorogenic dimerization reaction could also be utilized to determine amine concentration. In the present study, we attempted to develop a simple fluorometric assay for amines with benzofuran-2-boronic acid and palladium as reagents. Firstly, to investigate the applicability of the proposed fluorogenic reaction, the fluorescence of 19 kinds of typical amines were measured after the reaction with benzofuran-2-boronic acid in the presence of palladium catalyst. Next, we developed a simple and rapid microplate assay for TEA based on the proposed fluorogenic reaction.

Experimental
Materials and reagents

Benzofuran-2-boronic acid, diethylamine, pyrrole, ethanolamine and benzylethanolamine were purchased from Tokyo Chemical Industries (Tokyo, Japan). Palladium (II) acetate (Pd(OAc)_2), palladium (II) nitrate (Pd(NO_3)_2), hexylamine and choline chloride were purchased from Nacalai Tesque (Kyoto, Japan). Ethylamine, aniline, diphenylamine, trimethylamine, TEA, triphenylamine, acetylcholine chloride, L-carnitine, pyridine, piperidine, 1,3-diaminopropane, spermidine, spermine, tris{tris[3,5bis(trifluoromethyl)phenyl]phosphine}palladium (Superstable Pd(0)), tetrakis(triphenylphosphine)palladium(0) (Pd(PPh_3)_4), and bis(dibenzylideneaceton) palladium(0) (Pd(dba)_2) were purchased from Wako (Osaka, Japan). Acetonitrile (HPLC grade) and palladium (II) chloride (PdCl_2) were from Kanto Chemical (Tokyo, Japan). Water was distilled and passed through a Pure Line WL21P system (Yamamoto, Tokyo, Japan). All other chemicals were the highest purity and quality available. The stock solutions of amine were prepared in acetonitrile at the concentration of 500 µM each.

Fluorescence spectra of TEA after the reaction with benzofuran-2-boronic acid and Pd(OAc)_2

To 100 µL of TEA in acetonitrile, 100 µL each of 10 µM Pd(OAc)_2 and 5.0 mM benzofuran-2-boronic acid in acetonitrile were added, then the mixture was kept at room temperature for 15 min. After it was diluted 10 times with acetonitrile, the fluorescence emission spectrum excited at 315 nm was recorded on a RF-1500 spectrofluorometer (Shimadzu, Kyoto, Japan).

Fluorescence measurement by microplate reader

In each well of a 96-well black microplate (Corning, NY, USA), 50 µL of amines in acetonitrile, 50 µL of 10 µM Pd(OAc)_2 in acetonitrile, 50 µL of 5.0 mM benzofuran-2-boronic acid in acetonitrile were successively added. The microplate was immediately placed into a Spectra Max M5 microplate reader (Molecular Devices, Sunnyvale, CA, USA), and then was
shaken for 30 sec. After keeping the microplate for 15 min at room temperature in the microplate reader, the fluorescence was measured at excitation and emission wavelengths of 315 and 360 nm, respectively.

Results and discussion

Fluorescence response of amines after the reaction with benzofuran-2-boronic acid and Pd(OAc)$_2$

Figure 2 shows the emission spectra excited of benzofuran-2-boronic acid after the addition of TEA in the presence of Pd(OAc)$_2$. As the TEA concentration increased, the fluorescence with emission maxima at 360 nm increased at an excitation wavelength of 315 nm. The results meant that fluorogenic dimerization of benzofuran-2-boronic acid was promoted by addition of TEA depending to its concentration. Then, to investigate the applicable range of the proposed fluorogenic reaction, the fluorescence of 19 kinds of typical amines after the reaction with benzofuran-2-boronic acid and Pd(OAc)$_2$ were measured. As shown in Fig. 3, among the tested amines, TEA, trimethylamine, triphenylamine and diphenylamine provided a significant fluorescence enhancement resulted in signal-to-blank (S/B) ratio > 2. Also, it was found that TEA had the highest fluorescence intensity. Considering the results obtained by the present investigation, the proposed reaction would be useful to determine tertiary amines.

Fluorescence microplate assay for TEA

TEA is one of the volatile organic compounds and is used in variety of fields such as pharmaceuticals, agricultural chemicals, surfactants and raw materials for paints, etc.$^{9,10}$ It is known that TEA has a strong ammonia smell and can cause eye and lung irritation.$^{11,12}$ In actual, TEA is designated as a hazardous air pollutant. Although several gas chromatographic methods have been developed, these methods require relatively complicated instruments.$^{13-15}$ Although
fluorescence method has advantages in terms of simplicity, sensitivity and rapidness,\textsuperscript{16} TEA does not have intrinsic fluorescence, and it should be difficult to apply the fluorescence derivatization because TEA do not have derivatizable moiety.\textsuperscript{17} From these aspects, we attempted to develop a simple and rapid fluorescence microplate assay for TEA based on the promotion of dimerization of benzofuran-2-boronic acid.

In order to achieve high reactivity, the reaction conditions were optimized using a standard solution of TEA. The measurement results are shown as the average value of triplicate measurements. The optimum reaction time and temperature were 15 min and ambient temperature (25 °C), respectively, in consideration that the maximum S/B ratio could be achieved at these conditions (Fig. S1 and S2). The S/B ratio decreased with reaction time more than 15 min, because the signal with TEA reached maximum at approximately 15 min and kept contained, while blank without TEA slightly increased with increasing the reaction time. Then, the concentration of benzofuran-2-boronic acid was investigated over the range of 0-20 mM and 5 mM was selected because it gave the maximum S/B ratio (Fig. S3). The effect of Pd species on the fluorescence was investigated using superstable Pd(0), Pd(PPh\textsubscript{3})\textsubscript{4}, Pd(dba)\textsubscript{2}, Pd(NO\textsubscript{3})\textsubscript{2}, Pd(OAc)\textsubscript{2}, and PdCl\textsubscript{2}, because different Pd species could induce different dimerization reactivity (Fig. S4). Among the tested Pd species, the highest fluorescence intensity was obtained with Pd(OAc)\textsubscript{2}. Subsequently, the concentration of Pd(OAc)\textsubscript{2} was investigated over the range of 0-50 µM and the maximum S/B ratio was achieved with 10 µM Pd(OAc)\textsubscript{2}, and thus was selected (Fig. S5).

The calibration curve was prepared with standard TEA solution under optimized conditions. A good linear relationship ($r = 0.993$) between the TEA concentration and the fluorescence intensity was obtained in the range of 1 to 400 µM. The limit of detection (LOD) defined as the mean blank signal plus 3-times the standard derivation of the blank (blank + 3SD) was 0.091 µM. Table S1 summarizes the comparison of LOD values between the proposed assay and previously reported methods. The proposed assay was more than 10 times more sensitive than gas
chromatography with flame ionization detection, capillary electrophoresis, and chemiluminescence assay triggered by TEA free radical reduction of carbon dots. Also, the LOD of the proposed assay was similar to that of a fluorescence quenching assay based on the quenching of amphiphilic fluorescent micelles formed from polyethylene glycol and tetraphenylene ethylene.

The repeatability of the proposed assay was evaluated using three different concentrations (5, 50 and 400 µM) of standard TEA solution in the calibration range. The relative standard deviations (R.S.D) for intra-day (n = 5) assays were 4.4, 5.5 and 3.1%, respectively, and for inter-day (n = 5) assays were 6.2, 6.0 and 4.3%, respectively. Therefore, the sufficient repeatability of the proposed microplate assay could be proved.

**Conclusion**

We found that the fluorescence enhancement was observed from the solution of weakly-fluorescent benzofuran-2-boronic acid after the mixing with amine and palladium. The fluorescence enhancement could be attribute to the promotion of palladium catalytic fluorogenic dimerization of benzofuran-2-boronic acid by the addition of amine. The remarkable fluorescence was obtained from the mixture containing tertiary amines including TEA. Finally, the fluorogenic reaction could be successfully applied to develop a fluorescent microplate assay for TEA. Since the developed assay allowed a high-throughput measurement of TEA without derivatization, it should become a powerful tool to determine tertiary amines in environmental, biological and food samples,

**Supporting information**

This material is available free of charge on the Web at [http://www.jsac.or.jp/analsci/](http://www.jsac.or.jp/analsci/).
supporting information includes the results of optimization studies.
References

1. K. C. Nicolaou, P. G. Bulger, and D. Sarlah, Angew. Chem. Int. Ed., 2005, 44, 4442.
2. C. C. Johansson Seechurn, M. O. Kitching, T. J. Colacot, and V. Snieckus, Angew. Chem. Int. Ed., 2012, 51, 5062.
3. T. Higashijima, N. Kishikawa, and N. Kuroda, Anal. Sci., 2020, 36, 997.
4. S. Sakamoto, and I. Hamachi, Anal. Sci., 2019, 35, 5.
5. S. D. Walker, T. E. Barder, J. R. Martinelli, and S. L. Buchwald, Angew. Chem. Int. Ed., 2004, 43, 1871.
6. C. Torborg, and M. Beller, Adv. Synth. Catal., 2009, 351, 3027.
7. P. E. Broutin, I. Cerna, M. Campaniello, F. Leroux, and F. Colobert, Org. Lett., 2004, 6, 4419.
8. A. Higashi, N. Kishikawa, K. Ohyama, and N. Kuroda, Tetrahedron Lett., 2017, 58, 2774.
9. H. Xu, J. Ju, W. Li, J. Zhang, J. Wang, and B. Cao, Sens. Actuators B Chem., 2016, 228, 634.
10. D. Ju, H. Xu, Q. Xu, H. Gong, Z. Qiu, J. Guo, J. Zhang, and B. Cao, Sens. Actuators B Chem., 2015, 215, 39.
11. B. Akesson, I. Floren, and S. Skerfving, Br. J. Ind. Med., 1985, 42, 848.
12. L. Belin, U. Wass, G. Audunsson, and L. Mathiasson, Br. J. Ind. Med., 1983, 40, 251.
13. J. Namiesnik, A. Jastrzebska, and B. Zygmunt, J. Chromatogr. A, 2003, 1016, 1.
14. P. Neyer, L. Bernasconi, J. A. Fuchs, M. D. Allenspach, and C. Steuer, J. Clin. Lab. Anal., 2020, 34, e23062.
15. X. Li, Z. Zeng, J. Zhou, S. Gong, W. Wang, and Y. Chen, J. Chromatogr. A, 2004, 1041, 1.
16. C. Zhao, J. Shang, H. Li, C. Yang, Z. Li, and J. Ning, Anal. Sci., 2020, 36, 329.
17. Y. Zhou, H. Gao, F. Zhu, M. Ge, and G. Liang, J. Hazard. Mater., 2019, 368, 630.
18. A. R. Deshpande, G. Ramachandra, and R. S. Yamgar, *Eurasian J. Anal. Chem.*, 2012, 7, 43.

19. A. G. Lista, L. Arce, A. Rios, and M. Valcarcel, *Anal. Chim. Acta*, 2001, 438, 315.

20. H. Zhang, X. Zhang, and S. Dong, *Anal. Chem.*, 2015, 87, 11167.
Figure captions

Fig. 1 Fluorogenic dimerization of benzofuran-2-boronic acid by the addition of amine and palladium.

Fig. 2 The fluorescence emission spectra of benzofuran-2-boronic acid (5.0 mM) after the addition of different concentrations of TEA (0, 10, 30, 50, 80, 100, 200, 300, 400 and 500 µM) in the presence of Pd(OAc)$_2$.

Fig. 3 S/B ratio obtained from the fluorescence measurement of benzofuran-2-boronic acid after the addition of various amines and Pd(OAc)$_2$. The concentration of amines, benzofuran-2-boronic and Pd(OAc)$_2$ in acetonitrile were 500 µM, 5.0 mM and 10 µM, respectively. The reaction temperature was ambient temperature.
Fig. 1  Fluorogenic dimerization of benzofuran-2-boronic acid by the addition of amine and palladium.
Fig. 2  The fluorescence emission spectra of benzofuran-2-boronic acid (5.0 mM) after the addition of different concentrations of TEA (0, 10, 30, 50, 80, 100, 200, 300, 400 and 500 µM) in the presence of Pd(OAc)$_2$. 
Fig. 3  S/B ratio obtained from the fluorescence measurement of benzofuran-2-boronic acid after the addition of various amines and Pd(OAc)$_2$. The concentration of amines, benzofuran-2-boronic and Pd(OAc)$_2$ in acetonitrile were 500 µM, 5.0 mM and 10 µM, respectively. The reaction temperature was ambient temperature.
Graphical Index