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Design and synthesis of fluorescent reagents for selective detection of dopamine

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ABSTRACT

I report the development of (E)-2,2′-(5-(2-(4-(dicyanomethylene)-6-methyl-4H-pyran-2-yl)vinyl)-2-hydroxy benzylazanediyl)diacetic acid Fe(II) complex (**1-Fe**$^{2+}$), a fluorescent reagent that can be used to detect dopamine. **1-Fe**$^{2+}$ was constructed using the cyanopyranyl group as the fluorophore and an Fe^{2+} complex both as the ligand exchange site and fluorescence quenching moiety. In contrast to the weak fluorescence emission of **1-Fe**$^{2+}$ in the absence of dopamine, a much stronger fluorescence emission was observed following the addition of dopamine owing to the release of Fe^{2+} from compound **1**, which indicates significant fluorescence enhancement and the binding of Fe^{2+} to dopamine. The fluorescence intensities of the reagent were plotted as a function of the dopamine concentration and a good linear relationship was observed. The reaction of **1-Fe**$^{2+}$ with dopamine was not affected by the presence of foreign substances, thereby allowing for the highly selective detection of dopamine. The experimental results clearly showed that **1-Fe**$^{2+}$ is a good dopamine indicator, and it can be widely employed in dopamine detection protocols.

1. Introduction

Dopamine, a catecholamine compound, is a neurotransmitter that manages a wide range of cognitive functions, which include motivation, behavior, learning, and memory [1–3]. The amount of dopamine in the human brain is an important factor in various diseases, and is one of the markers used in the diagnosis of several conditions related to neurotransmitters, including Parkinson's disease [4]. The pathways that transmit dopamine in the brain are known to modulate the behavior of other neural systems. Therefore, imbalances in dopamine levels are believed to lead to behavioral disorders such as schizophrenia, autism, and depression. Furthermore, the degradation of dopaminergic pathways plays a major role in the pathology of neurodegenerative disorders such as Alzheimer's, Huntington's, and Parkinson's diseases, which are attracting considerable attention because these diseases are increasingly affecting the general population [5,6]. Consequently, there is a strong need to develop efficient and rapid methods that can be used to selectively determine and continuously sense changes in the dopamine levels.

Fluorescence spectrometry is a conventional and highly sensitive analytical method. Fluorescent probes that exhibit a spectral response upon binding to ions and neutral organic or inorganic molecules have enabled researchers to investigate changes in free guest ions or changes in the concentrations of molecules using fluorescence microscopy, flow cytometry, and fluorescence spectroscopy [7–11]. Previous reports on fluorometric determination of dopamine focused on the native fluorescence properties of the analytes, fluorescence quenching efficiency arising from the reaction of dopamine with HRP, quantum dot, derivatization by ethylenediamine following oxidation using mercury(II) nitrate, as well as a ligand exchange method involving the use of a calcein blue-Fe^{2+} complex [12–19]. However, these methods are not selective toward dopamine and further separation techniques such as ion exchange separation, thin layer chromatographic separation, or HPLC were additionally required for the selective determination of dopamine. Moreover, dopamine detection using the calcein blue-Fe^{2+} complex indicates small fluorescence enhancement after addition of small amount of dopamine.

The requirements that we considered while designing a fluorescent reagent to detect dopamine include the production of weak to strong fluorescence following reaction with dopamine, which may eliminate background noise and result in the highly sensitive detection of dopamine; high selectivity and sensitivity to dopamine; reduced interference from foreign substances.

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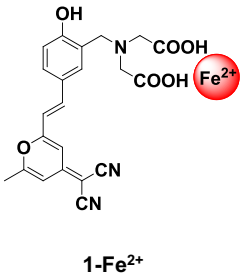


1-Fe^{2+}

Fig. 1. Chemical structure of the fluorescent reagent **1-Fe**$^{2+}$.

In previous studies, novel fluorescent reagents have been developed to detect biological molecules, which are sensitive to changes in the external environment; the quantum yields of these molecules generally increase as the environment becomes hydrophobic, resulting in dramatic fluorescence spectral changes [20–26]. To take advantage of this phenomenon, novel fluorescent probes were subjected to external environments; the results showed that their quantum yields responded linearly to the concentration of target compounds.

In this study, I propose a technique that combines fluorescent molecular probes, using the cyanopyranyl group as the fluorescent emitter and an imino-di-acetic acid-Fe^{2+} complex both as a fluorescent quencher and a ligand exchange moiety for the detection of dopamine. The chemical structure of the molecular probe (compound **1** and its Fe^{2+} complex, **1-Fe**$^{2+}$) is shown in Fig. 1. Compound **1** and **1-Fe**$^{2+}$ were effectively synthesized, and the interactions of these molecules with dopamine in a homogenous solution system were investigated. Experimental results clearly indicate that the reagents developed in this study act as good fluorescent indicators and they can be widely used in the detection of dopamine.

2. Materials and methods

2.1. General information

All chemicals used were of analytical reagent grade and were purchased from the Tokyo Chemical Industry (TCI, Tokyo, Japan), Wako Pure Chemical Industries, Ltd. (Osaka, Japan), and Sigma-Aldrich. Absorption spectra were recorded at 25 °C using a UV/visible spectrophotometer (V-670, JASCO), and fluorescence spectra were recorded at 25 °C using a JASCO FP-6500 fluorophotometer. ^{1}H NMR spectra were recorded using a Bruker AV500 M spectrometer.

2.2. Synthesis of fluorescent reagent for the detection of dopamine

The scheme for the synthesis of fluorescent reagents is illustrated in Scheme 1, and individual synthetic protocols are detailed below.

2.2.1. *Diethyl-2,2′-(5-formyl-2-hydroxybenzylazanediyl)diacetate*

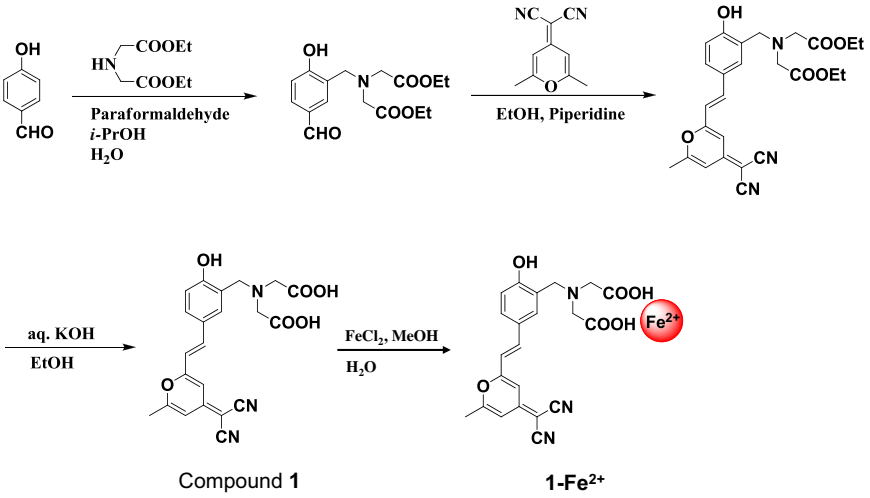
Diethyl iminodiacetate (1.9 g, 10.0 mmol) was added to a solution of paraformaldehyde (1.3 g) in 30 mL isopropyl alcohol and 50 mL H_2O, and the solution was stirred for 45 min at 80 °C under N_2 atmosphere. Following the addition of 4-hydroxybenzaldehyde (1.0 g, 8.2 mmol), the reaction mixture was refluxed for 24 h and the solvent then removed in vacuo. The resulting residue was dissolved in chloroform, washed with water, and dried over Na_2SO_4. Following the removal of the solvent, the product was purified by column chromatography (SiO_2; $CHCl_3$:MeOH = 200: 3, v/v). The yield of the process was 75%.

^{1}H NMR ($CDCl_3$, 500 MHz, r.t., TMS, δ/ppm) 1.24 (6H, t), 3.53 (4H, s), 4.04 (2H, s), 4.21 (4H, q), 6.99 (1H, d), 7.57 (1H, s), 7.75 (1H, d), 9.81 (1H, s) 10.54 (1H, bs).

2.2.2. *(E)-diethyl-2,2′-(5-(2-(4-(dicyanomethylene)-6-methyl-4H-pyran-2-yl)vinyl)-2-hydroxybenzyl azanediyl)diacetate*

To a solution of diethyl 2,2′-(5-formyl-2-hydroxybenzylazanediyl)diacetate (0.5 g, 1.6 mmol) in 40 mL ethanol, 4-(dicyanomethylene)-2,6-dimethyl-4H-pyran (0.3 g, 1.6 mmol) and piperidine (0.2 g, 1.7 mmol) were added, and then refluxed for 12 h under N_2 atmosphere. After removing the solvent in vacuo, the residue was dissolved in $CHCl_3$, washed with H_2O, and dried over Na_2SO_4. The solvent was evaporated *in vacuo*, and the product was then purified by column chromatography (SiO_2; n-hexane:ethyl acetate = 3:2 v/v → $CHCl_3$). The yield of the process was 84%.

^{1}H NMR ($CDCl_3$, 400 MHz, r.t., TMS, δ/ppm) 1.29 (6H, t), 2.38 (s, 3H), 3.54 (4H, s), 4.02 (2H, s), 4.22 (4H, q), 6.50-6.53 (2H, m), 6.62 (1H, s), 6.94 (1H, d), 7.18 (1H, s), 7.40-7.42 (2H, m), 9.95 (1H, bs).



Compound **1** **1-Fe^{2+}**

Scheme 1. Synthetic method of fluorescent reagent (**1-Fe**$^{2+}$).