A new approach for turn-on fluorescence sensing of L-DOPA†

Lu Wang, Dongdong Su, Stuart N. Berry, Jungyeol Lee and Young-Tae Chang

Dopamine is known as an important neurotransmitter of the human central nervous system and affects many brain functions and behavioral responses. Lack of dopamine in the brain may lead to neurological disorders, such as schizophrenia and Parkinson’s disease. The precursor to dopamine, L-DOPA is converted to dopamine via a metabolic pathway and, unlike dopamine, L-DOPA has the ability to cross the protective blood–brain barrier. This has allowed the external use of L-DOPA due to its reduction properties; this sensing mechanism was fully confirmed by mechanistic studies. Furthermore, Resa-Sulf was successfully utilized to quantitatively detect L-DOPA concentrations from a commercially available source.

Up to now, only limited numbers of small molecule fluorescent sensors for L-DOPA have been reported. Furthermore, most of these small molecule sensors rely on quenching or turn-off sensing mechanisms. It is known that turn-on sensors have comparatively better sensitivity, higher resolution as well as lower potential errors than turn-off sensors. A good turn-on sensor, therefore, may have unique potential for the future real-time in vivo or ex vivo imaging of L-DOPA.

Herein, we have demonstrated a new approach for turn-on fluorescence sensing of L-DOPA by using its reduction properties. The tendency of L-DOPA to donate an electron in solution can be exploited by using it as a reductant in a redox reaction and based on this, we sought to develop a redox reaction based turn-on fluorescence sensor for L-DOPA. The major advantage of redox reaction sensing is that the molecular recognition events can occur in a short time span, with observable changes in color and/or fluorescence intensity. Furthermore, quantitative detection can be easily achieved because of the stoichiometric nature of the redox reaction. Up to now, according to the selected redox reaction system, several examples based on redox reactions have been developed for appropriate applications.\textsuperscript{17–20}

Catecholamines with reduction properties can be oxidized in either water or buffer solution. This property has been previously applied in methods based on the electrochemical analysis of dopamine.\textsuperscript{21–23} Inspired by this detection approach, we report a novel design strategy for the preparation of fluorescent sensors for the detection of L-DOPA. To the best of our knowledge, this is the first fluorescent sensor for L-DOPA based on a redox reaction. To demonstrate the application of this redox reactive approach, we employed a resazurin dye as our signal reporter not only due to its sensitive and simple mechanism of action, but also due to its unique oxidative and electron dependent optical properties. Our previous work has also shown that resazurin-based dyes can be utilized in redox reaction based sensors.\textsuperscript{25}

Scheme 1 indicates the structures of the fluorescent sensors investigated in this study and the proposed sensing approach. Resazurin-based sensors (Resa-Sulf and Resa-Con) are shown as self-quenching fluorophores, however, upon addition of L-DOPA,
A redox reaction occurs and \(1\text{-DOPA}\) as the reductant reduces the weakly fluorescent resazurin fluorophore, leading to deoxygenation of the \(N\)-oxide group, producing the resultant resorufin structures as strongly fluorescent products. The synthesis of these sensors is described in Fig. S1 (ESI†), and all sensors were fully characterized by \(^1\)H NMR, \(^{13}\)C NMR and HRMS (shown in the ESI†).

To obtain the optimum sensing conditions, the fluorescence response of \(\text{Resa-Con}\) to different analytes as functions of time and pH was systematically studied in DMSO/PBS buffer solution (v/v = 1:1). As shown in Fig. S2 (ESI†), \(\text{Resa-Con}\) is almost non-fluorescent over a large pH range from 4.3 to 11.2. Upon treatment with \(1\text{-DOPA}\), as well as other catecholamines, the fluorescence is enhanced. Furthermore, the fluorescence enhancement is greater in weakly basic solutions compared to under acidic conditions. By evaluating the reaction rate, upon addition of analytes at pH 11.2, the fluorescence is almost saturated within 3 min of analyte addition, and a color change from red to yellow is observed. This makes it possible to detect \(1\text{-DOPA}\) via the naked eye or under irradiation using a UV lamp. These results are consistent with a previous study which shows that the absorption and fluorescence properties of resazurin and resorufin are dependent on pH, and that the fluorescence is enhanced at higher pH. \(^{26–28}\)

Unfortunately, under these conditions, \(\text{Resa-Con}\) does not show perfect selectivity towards other catecholamines, such as dopamine, epinephrine and norepinephrine. This result is not surprising, because these three types of catecholamines share similar functional groups. \(^{26–28}\)

In order to reduce the fraction of DMSO in the sensing system, we prepared \(\text{Resa-Sulf}\) to improve aqueous solubility. By comparing the fluorescence responses of \(\text{Resa-Con}\) and \(\text{Resa-Sulf}\) towards \(1\text{-DOPA}\), we found that with 50% DMSO as a co-solvent, the maximum fluorescence intensities of the two sensors were essentially the same in the presence of \(1\text{-DOPA}\); however, in 1% DMSO/PBS buffer, the highest fluorescence intensity of the water-soluble fluorescent sensor, \(\text{Resa-Sulf}\), is almost 7 times that of non-water-soluble \(\text{Resa-Con}\) after 2 min of incubation. This provides higher sensitivity and a larger response range in the aqueous environment (Fig. S3, ESI†). Clearly, aggregation of \(\text{Resa-Con}\) was observed in 1% DMSO/PBS buffer (Fig. S4, ESI†). Considering the fast response and high sensitivity between \(\text{Resa-Sulf}\) and \(1\text{-DOPA}\), 1% DMSO/PBS buffer at pH 11.2 was selected as the sensing solvent for further experiments. Subsequently, the time-dependent fluorescence changes of \(\text{Resa-Sulf}\) (50 \(\mu\)M) in the presence of \(1\text{-DOPA}\) (0–100 \(\mu\)M) were investigated. This experiment showed that the enhanced fluorescence intensity saturates within nearly 2 minutes of incubation (Fig. S5, ESI†).

Encouraged by the fast response, we then tested the reactivity of \(\text{Resa-Sulf}\) upon addition of \(1\text{-DOPA}\) in PBS with an incubation time of 2 min. The spectral changes of \(\text{Resa-Sulf}\) during the titration with \(1\text{-DOPA}\) are shown in Fig. 1. Upon addition of increasing concentrations of \(1\text{-DOPA}\), the maximum absorption band changed from 530 nm to 480 nm, meanwhile, the fluorescence band centered at 615 nm blue-shifts to 580 nm, which results from the redox reaction between \(\text{Resa-Sulf}\) and \(1\text{-DOPA}\) (movie, ESI†). Pleasingly, the changes in fluorescence intensities of \(\text{Resa-Sulf}\) (50 \(\mu\)M) showed a linear calibration response to \(1\text{-DOPA}\) concentrations from 0 to 50 \(\mu\)M with the coefficient of determination \(R^2 = 0.9997\) (Fig. 1C). Furthermore, to test the sensitivity of \(\text{Resa-Sulf}\), similar fluorescence titrations were performed with \(\text{Resa-Sulf}\) at 1 \(\mu\)M, and the fluorescence response to \(1\text{-DOPA}\) also showed a good linear relationship \((R^2 = 0.9926)\) even at very low \(1\text{-DOPA}\) concentrations ranging from 0 to 0.8 \(\mu\)M (Fig. 1D). Notably, the detectable concentration of \(1\text{-DOPA}\) can be as low as 0.01 \(\mu\)M. More importantly, as one of the advantages of redox reaction based sensing, the ratio of the sensor to analyte can be easily identified based on the nature of the redox reaction. As expected, the fluorescence of \(\text{Resa-Sulf}\) (20 \(\mu\)M) increased to the maximum level at a concentration of \(1\text{-DOPA}\) of 20 \(\mu\)M in 10 min, which suggests that the ratio between \(\text{Resa-Sulf}\) and \(1\text{-DOPA}\) is 1:1 (Fig. S6, ESI†). Furthermore, Job’s plot analysis also confirmed a 1:1 reaction stoichiometry (Fig. S7, ESI†). \(^{29}\) The fast response and the clear reaction ratio suggest that \(\text{Resa-Sulf}\) has good potential for application in the quantitative detection of \(1\text{-DOPA}\) concentrations.

In order to examine the selectivity of \(\text{Resa-Sulf}\) towards \(1\text{-DOPA}\), the fluorescence changes of \(\text{Resa-Sulf}\) in the presence of different redox reagents, such as NADH, catechol and amino acids, were examined by monitoring the changes of the peak maxima at 570 nm. With the exception of dopamine, epinephrine
and norepinephrine, which share similar structures with l-DOPA, all other species showed no response to Resa-Sulf. Upon addition of an equivalent amount of l-DOPA to each competitive species, the fluorescence enhancement is recovered (Fig. 2). In particular, Resa-Sulf does not respond to catechol, probably due to the differences in the electrochemical properties to catecholamines. These results indicated that Resa-Sulf has relatively good selectivity for l-DOPA and catecholamines over other biologically relevant redox regents. Therefore, Resa-Sulf shows potential application in l-DOPA analysis in the absence of catecholamines. It should be noted that only in the presence of dopamine is a similar fluorescence response observed: epinephrine and norepinephrine give small fluorescence maxima compared to in the presence of l-DOPA. The reaction rate constants are shown in Fig. S8 (ESI†). Considering the complexity of the intracellular environment, it is difficult to use this probe for neurotransmitter imaging by simple incubation; however, it still has high potential for cell imaging if the probe can be delivered into specific organelles in neuronal cells.30

To obtain more detailed insights into the sensing mechanism, HPLC-MS was employed to characterize the reaction between Resa-Sulf and l-DOPA.31 The Resa-Sulf samples were injected for HPLC before and after 2 min of incubation of l-DOPA. The results from this HPLC-MS test showed that the signal corresponding to Resa-Sulf at 8.1 min disappeared and a new peak corresponding to the reduction product Reso-Sulf at 8.5 min emerged (Fig. 3). The results were further confirmed by mass spectral analysis. The signal at 8.1 min shows characteristic mass information for Resa-Sulf, while the new peak at 8.5 min shows mass information for the reduction product Reso-Sulf (Fig. S9, ESI†).

To further confirm the proposed sensing mechanism, the reaction of Resa-Sulf with l-DOPA was also analyzed by 1H NMR spectroscopy. As shown in Fig. 4, upon the addition of l-DOPA, the major peaks of Resa-Sulf ranging from 6 ppm to 8 ppm disappeared, while new peaks corresponding to the Reso-Sulf product clearly emerged. In particular, the two doublets at 8.02 ppm and 7.98 ppm which correspond to the proton resonances on either side of the N-oxide group undergo a large upfield shift to 7.55 ppm and 7.38 ppm respectively in the reduced species due to the loss of deshielding effects. Furthermore, Reso-Sulf was successfully isolated and fully characterized in high yield after the reaction of Resa-Sulf with l-DOPA (see the ESI† for synthesis and characterization). Taken together, these results clearly confirm the reductive sensing mechanism of l-DOPA with Resa-Sulf.

Our goal is to develop a simple and reliable sensing method for the quantitative detection of l-DOPA in real samples. Many health care products containing l-DOPA are sold as mood enhancers or to improve stress responses, and also as drugs for the treatment of Parkinson’s disease and dopamine-responsive dystonia; however, overuse of l-DOPA may cause serious side effects, such as nausea, vomiting, strong headaches, and disruption of sleep cycles.32,33 In order to quantitatively detect l-DOPA concentrations, we applied Resa-Sulf to analyze l-DOPA in real samples. Standard calibration curves were developed using fluorescence and HPLC methods with known, different concentrations of pure l-DOPA (Fig. S10 and S11, ESI†).33 By comparing the actual l-DOPA amount acquired from HPLC and fluorescence measurements, we found that the calculated data for l-DOPA from different methods correlated very well, thus confirming that Resa-Sulf is feasible for practical determination of L-DOPA in real samples. A commercially available source, DOPA Mucuna Veg Capsules, was purchased from Now Foods and used without further treatment. The DOPA capsule was dissolved and diluted before analysis by treatment with Resa-Sulf. Table 1 shows that Resa-Sulf can be used to determine the concentration of l-DOPA in commercial...
samples with good recovery. The level of L-DOPA was calculated to be 13.09% and 12.85% based on Resa-Sulf and HPLC measurements respectively (Fig. S12, ESI†), which agrees well with the content declared by the company (15%). Thus, Resa-Sulf can be utilized as a simple tool to accurately quantify L-DOPA concentrations in commercially available health care products or L-DOPA containing plants.

In summary, by exploiting the reductive properties of L-DOPA, we have found a simple, rapid turn-on sensor for the quantitative detection of L-DOPA for the first time. The water-soluble sensor Resa-Sulf shows high selectivity and fast response towards L-DOPA, and the nature of the redox reaction makes it a good, practical tool for the quantitative detection of L-DOPA in real samples. This approach was successfully applied in the detection of a commercially available source of L-DOPA with a simple operation process. HPLC-MS and 1H NMR studies provided a strong support for the mechanism of L-DOPA detection by Resa-Sulf. Therefore, this new approach not only provides an efficient tool for L-DOPA detection, but also offers a novel design strategy for the future development of other catecholamines sensors.

We gratefully acknowledge the intramural funding from A*STAR (Agency for Science, Technology and Research, Singapore) Biomedical Research Council and National Medical Research Council grant (NMRC/TCR/016-NNI/2016).

Conflicts of interest

There are no conflicts to declare.

Notes and references


3 K. J. Broadley, Pharmacol. Ther., 2010, 125, 363–375.


