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Rapid and sensitive fluorescent probes for monoamine oxidases B to A at low concentrations

Yexiang Zhang, Yufang Xu, Shaoying Tan, Lin Xu, Xuhong Qian *

State Key Laboratory of Bioreactor Engineering, Shanghai Key Laboratory of Chemical Biology, East China University of Science and Technology, Shanghai 200237, China

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ABSTRACT

A new monoamine oxidase (MAO) fluorescent probe that displays response for both monoamine oxidase type-A (MAO-A) and monoamine oxidase type-B (MAO-B) is reported. This sensing platform does not require any additional enzymes or reagents. Furthermore, the probes have better sensitivity and shorter response time by comparison with similar probes, and have potentials in differentiation of MAO-A and MAO-B upon further optimization.

Monoamine oxidases (MAOs) are found in the mitochondria of most mammalian cells in the central nervous system and peripheral organs which catalyze the oxidative deamination of their substrates.[1] These enzymes play a vital role in neurotransmitter amine deactivation, catabolism, and metabolism.[2] Abnormal levels of MAOs in humans are associated with depression, schizophrenia, substance abuse, attention deficit disorder, migraines, irregular sexual maturation, and other diseases.[3]

In humans, there are two forms of MAO which are designated as monoamine oxidase type-A (MAO-A) and monoamine oxidase type-B (MAO-B).[4] Their differences include the amino acid sequences, tissue distribution, three-dimensional structure, inhibitor selectivity, and substrate preferences.[5] For these reasons, they show different substrate and inhibitor specificities: serotonin is metabolized by MAO-A, which is inhibited by low concentrations of clorgyline; while dopamine is metabolized by MAO-B, which can be inhibited by nanomolar concentrations of selegiline (*l*-deprenyl).[6] Within the human brain, MAO-A and MAO-B are expressed to varying degrees depending upon the cell type.[7] Because of the medical importance of MAOs, the MAO inhibitors have been developed as drugs for treating clinical depression, Parkinson's disease, Alzheimer's disease, and cerebral ischemia and strokes.[8] Especially, the inhibitors specific for MAO-B have been developed using pyrrole derivatives, 3-arylcoumarins derivatives.[9]

The fluorescence detection method is preferred for its satisfactory sensitivity, facile operation, and real-time monitoring in environmental and biological samples. The fluorescent detection for MAOs has become a hot topic these years. For example, Sames et al. have developed a sensor for both MAO-A and MAO-B, based on a coumarin system, fluorescence of which can be recovered upon formation of an indole via an aldehyde intermediate.[10] Chang et al. have also developed a probe for MAOs based on a tandem amine oxidation/elimination mechanism using resorufin as a fluorescent reporter.[11] Ahn et al. have developed a fluorescent probe for MAOs based on two-photon absorbing material.[12] Zhu et al. have done a lot of work for sensing MAO-B lately.[13] Other groups have also developed some probes for MAO by using peptide-PNA or ^{19}F magnetic resonance imaging.[14]

However, the sensitivity and response time of known probes, which are important for future medical applications, were still needed to be improved. Also, most of them could not distinguish MAO-A and MAO-B very well. Here, a new fluorescent probe **CR1** for MAO, which can distinguish MAO-A and MAO-B to a certain extent, has been developed. Furthermore, the sensitivity of the probe for MAOs has been enhanced which is evaluated by the response time and the concentration test for MAOs.

Matos and his group have developed a selective MAO-B inhibitor whose result showed that the introduction of a *p*-methoxy group increases the inhibitory activity.[15] Inspired by this inhibitor and the known MAO fluorescent probes;[10–13] we design a coumarin probe with a *p*-methoxy group, and a propyl linker between the amine substrate and fluorophore (Scheme 1). Based on such a proposal, fluorescence of the probe would be quenched because of the free amine of the aniline and the rotation of the linker, while the fluorescence of the coumarin system would be recovered upon MAO in buffer, since the amine was oxidized by MAO to afford iminium or aldehyde intermediates that would undergo β-elimination,

* Corresponding author. Tel.: +86 21 64251399; fax: +86 21 64252603.
E-mail address: xhqian@ecust.edu.cn (X. Qian).

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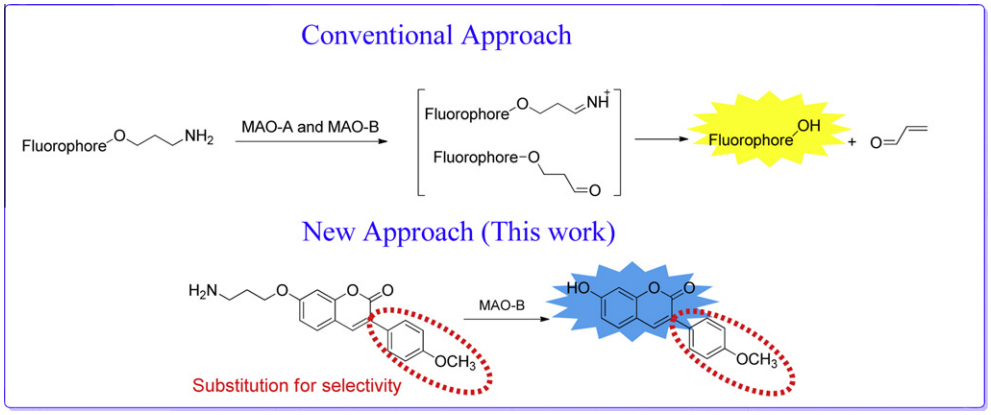
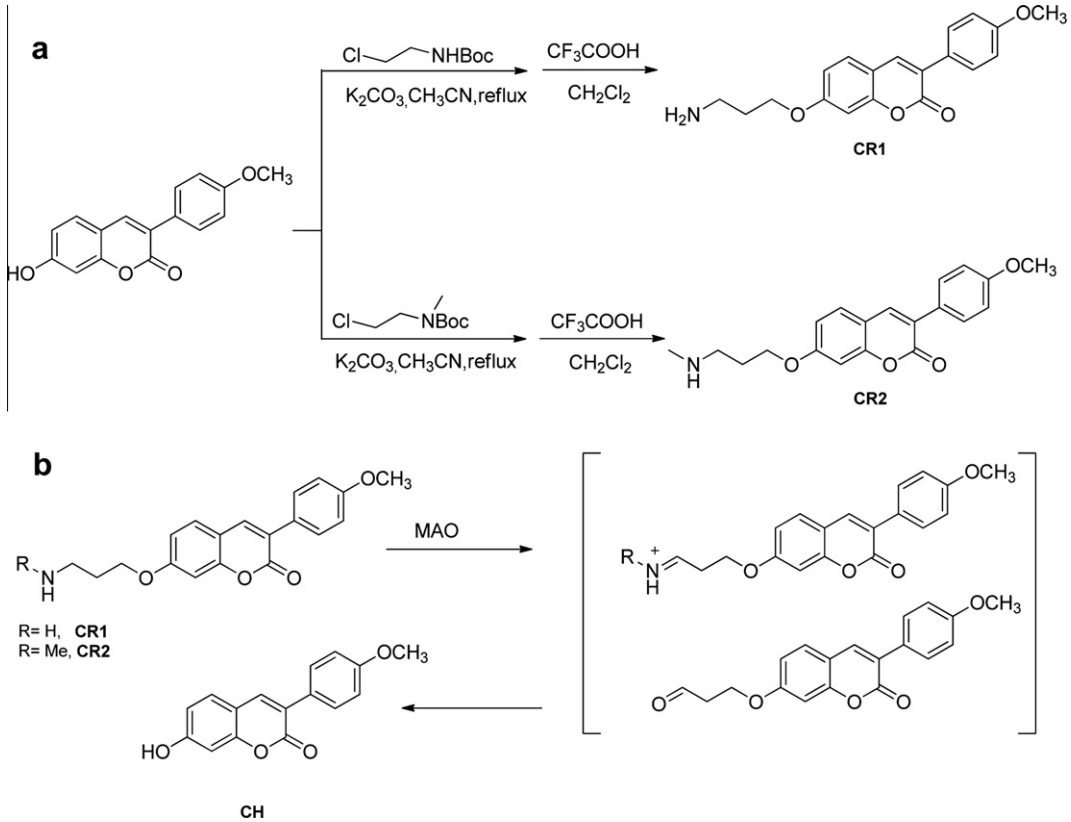


Scheme 1. The design of a new MAO fluorescent probe **CR1**.

and formed fluorescent-hydroxyl coumarin as product. In addition, the *p*-methoxy group was introduced to enhance the sensitivity to MAO-B. We anticipate that the intensity of the fluorescence will be enhanced more upon MAO-B than A at low concentrations, and furthermore, the fluorescence intensity of the probe will increase with increasing concentration of MAO-A and MAO-B.

Scheme 2a outlines the synthesis of probes **CR1** and **CR2** and the principle of its design. The hydroxyl coumarin was easily synthesized according to the literature using the Pechmann reaction.[16] Reactions of *N*-methyl-3-chloropropylamine or 3-chloropropylamine in the presence of $(Boc)_2O$ and potassium carbonate furnished the amines in a total yield of 90% and 92%, respectively. Deprotection of Boc after the reaction of the protected amine and hydroxyl coumarin, the probes **CR1** and **CR2** were obtained in a total yield of 61% and 74%, respectively. The probe was characterized by ^1H NMR, ^{13}C NMR, and mass spectrometry. The principle of sensing MAO is presented at Scheme 2b. At low concentrations, the probe **CR1** is oxidized by MAO-B but not MAO-A to form iminium or aldehyde intermediates that would undergo β-elimination, and fluorescent-hydroxyl coumarin is formed as product which enhances the intensity of fluorescence. At higher concentration, the selectivity of **CR1** is lost and turn-on fluorescence is observed for both MAO-A and B.

Spectroscopy determination of **CR1** and **CR2** was carried out in HEPES buffer (100 mM pH 7.4 with 5% glycerol and 1% DMSO at 37 °C). The spectrum showed that **CR1** and **CR2** featured one obvious absorption band at 345 nm and emission band at 465 nm which were characteristic of the coumarin core. The effect of pH on fluorescence properties of the probes **CR1** and **CR2** was determined. As shown in Figure 1, the spectra of emission remained



Scheme 2. (a) The synthesis route of probes **CR1** and **CR2**; (b) the principle of the sensing process.

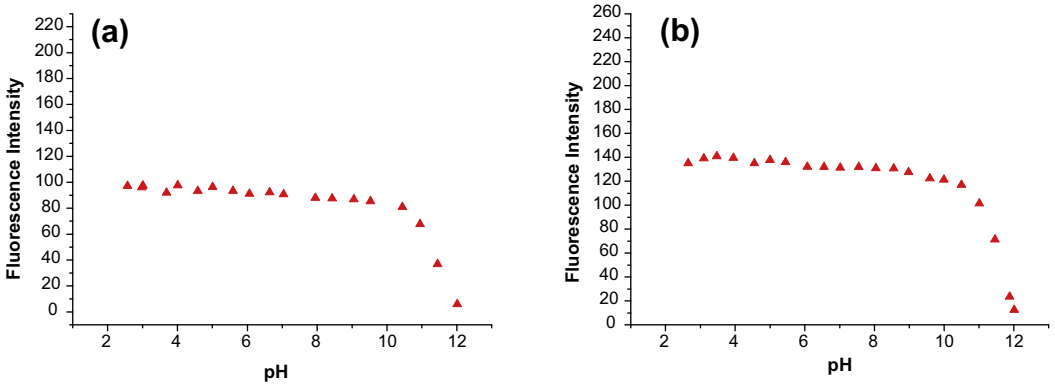


Figure 1. (a) The pH dependence of the fluorescence intensity of **CR1** in H_2O. (b) The pH dependence of the fluorescence intensity of **CR2** in H_2O.

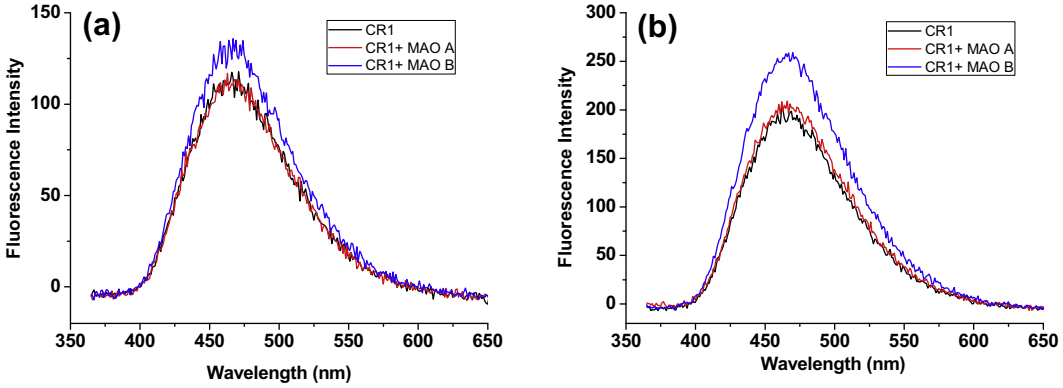


Figure 2. (a) Emission spectra of **CR1** (1 μmol/L), **CR1**+MAO-A (5 μg/ml), and **CR1**+MAO-B (5 μg/ml). Reactions were performed at 37 °C in enzyme assay buffer (100 mM HEPES, pH 7.4 with 5% glycerol and 1% DMSO). (b) Emission spectra of **CR1** (2 μmol/L), **CR1**+MAO-A (5 μg/ml), and **CR1**+MAO-B (5 μg/ml). Reactions were performed at 37 °C in enzyme assay buffer (100 mM HEPES, pH 7.4 with 5% glycerol and 1% DMSO).

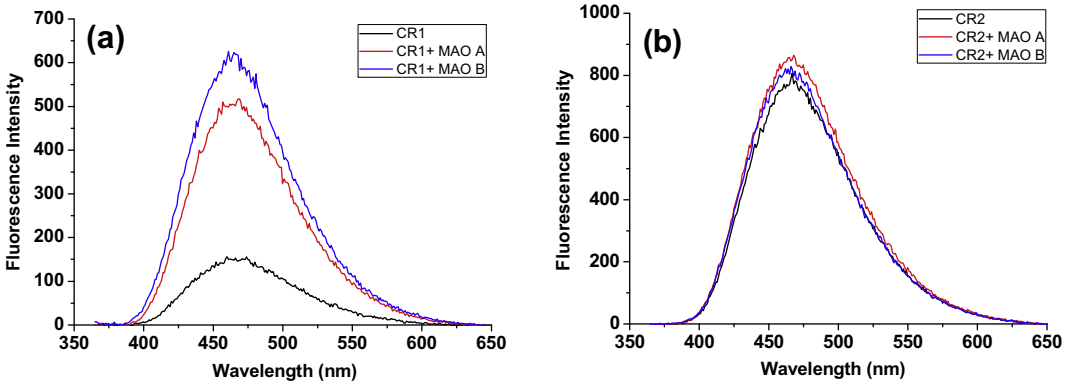


Figure 3. (a) Emission spectra of **CR1** (1 μmol/L), **CR1**+MAO-A (50 μg/ml), and **CR1**+MAO-B (50 μg/ml). Reactions were performed at 37 °C in enzyme assay buffer (100 mM HEPES, pH 7.4 with 5% glycerol and 1% DMSO). (b) Emission spectra of **CR2** (1 μmol/L), **CR2**+MAO-A (50 μg/ml), and **CR2**+MAO-B (50 μg/ml). Reactions were performed at 37 °C in enzyme assay buffer (100 mM HEPES, pH 7.4 with 5% glycerol and 1% DMSO).

stable with the pH from 2 to 10. This indicates that the probes could be used for sensing MAO in a wide range of pH. Then, we determined the quantum yield of the two compounds. As we expected before, the probes **CR1** and **CR2** have very weak fluorescence ($\Phi = 0.11$) compared with the hydroxyl coumarin ($\Phi = 0.49$), which meant that the fluorescent intensity of the probe should increase upon the addition of MAOs.

Then, we wanted to find out whether there was different response after adding MAO-A and MAO-B at low concentrations. First, we tested the probe **CR1** with MAO-A and MAO-B. We found that the fluorescence intensity of **CR1** both increased 29% and 31% with MAO-B at the concentrations of 1 μmol/L and 2 μmol/L but almost no increase with MAO-A (Fig. 2).

Later, we tested the fluorescence intensity of the probe **CR1** response to MAO-A and MAO-B at the higher concentration. As shown in Figure 3a, the fluorescence intensity of probe **CR1** was increased from 140 to 500 and 600, respectively. The large responses for the fluorescent probe were due to the formation of fluorescent-hydroxyl coumarin after β-elimination of the probe. Here, the fluorescent intensity of product, fluorescent-hydroxyl coumarin (**CH**)

was also determined lonely (see Supplementary data). The intensity of **CH** was 650, which was almost the same with the probe after addition of MAO.

Interestingly, the result of fluorescent test for **CR2** was very different from what we expected before. The structure of **CR2** is almost the same with **CR1** but a substitution of methyl, according to our assumption, the response of **CR2** upon addition of MAO is similar to **CR1**. (Fig. 3b) But to our surprise, the fluorescence intensity was almost of no change with MAO-A and MAO-B. The reason maybe the affinity of **CR2** with MAOs was much weaker than **CR1**.

As we mentioned before, although the selectivity is still needed to be improved, the sensitivity of this probe was increased more compared with the other known probes. The concentration of this probe in the test was 1 μmol/L and 2 μmol/L which were lower than the most probes, even the lowest concentration of the known probe (5 μmol/L). On the other hand, the response time of sensing for MAO was tested which is another indicator of the sensitivity of the probes. The result showed that after addition of the same concentration of MAO A (50 μg/mL), the fluorescence intensity of the probe **CR1** (5 μmol/L) peaked at 1 min, which was much shorter than the known probe (7.6 μmol/L, 10–60 min).[11] (See Supplementary data)

In conclusion, we have reported a novel and sensitive monoamine oxidase probe. Interestingly, this was the first time to report a probe whose intensity could be increased by MAO-B but not MAO-A at a lower concentration. The detailed reason maybe the addition of the bulky group into the fluorophore which changes the affinity with the MAO. Furthermore, the probe was a turn-on probe for both MAO-A and MAO-B at high concentration. On the other hand, the sensitivity of the probes was much enhanced than that of the known probe. According to our research and the known probes,[10–13] a molecule with a propyl linker between the amine substrate and fluorophore can be used as a selective, sensitive fluorescent probe for MAO. So, this might be a good platform to build a MAO-A and MAO-B distinguishable fluorescent probe.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2012.09.074.

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