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Highly selective and sensitive fluorescence turn-on probe for proline

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ABSTRACT

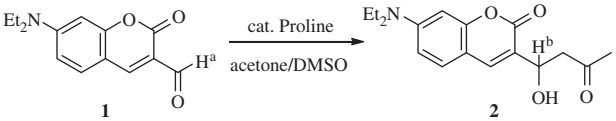
A weakly fluorescent coumarinyl aldehyde was transformed into a strongly fluorescent aldol product by a catalytic amount of proline. The aldehyde probe has shown a highly selective fluorescence turn-on response toward proline over other amino acids with micromolar sensitivity.

Over the past years, extensive research about amino acids has been carried out for the purpose of separation,[1] deracemization,[2] and catalytic synthesis[3] of the highly charged and water-soluble species. Recently, several probes for the amino acids were developed as a universal chirality sensor[4] or a universal receptor for α-amino acids.[5] Probes, which are selective for certain amino acids such as aromatic amino acid,[6] aspartate,[7] or cysteine,[8] have also been reported.

Proline, that is frequently found in the β turn structures of folded protein chains,[9] is one of the interesting amino acids. Higher level of proline in the blood causes a hyperprolinemia (HP-II), whose proline levels are between 10 and 15 times higher than normal, and provokes seizures or intellectual disability.[10] Thus, noninvasive analysis of the amounts of proline and its metabolites in urine is attracting a considerable interest.[11] In spite of the biological and pathological importance, few luminescent probes for proline were reported,[12] partly due to the weak nucleophilicity of the amino group of proline or partly due to the weak coordination ability of the secondary amino group of proline compared to the primary amino group of other amino acids. To overcome these flaws of proline, we used a secondary amine-mediated reaction for the proline-selective probe. It has been well known that proline catalyzes an aldol reaction.[13] The intensive mechanistic study for the organic catalyst has revealed the aldol reaction proceeds via an on-cycle enamine formation together with a dual role of water.[14] Motivated by the proline-catalyzed aldol reaction, we prepared an aldehyde-functionalized coumarin (**1**) as an aldol reactant according to the literature procedure.[15] If the aldehyde fluorophore and the enamine or enol counterpart combine to produce an aldol product, the fluorescence of the probe will be significantly affected owing to the change in the hybridization of carbon near the fluorophore. For a fluorescence turn-on probe, a weakly fluorescent coumarinyl aldehyde (**1**) was utilized as an appropriate chemodosimeter because the quencher, sp^2-hybridized carbonyl group is transformed into a sp^3-hybridized alcohol by the aldol reaction (Scheme 1). Herein, we report a highly selective and sensitive fluorescence turn-on probe for proline by an aldol reaction.

The chemical transformation of **1** into the aldol product was monitored after the addition of L-proline (L-Pro) to **1** in DMSO-d_6/acetone-d_6 (4:1, v/v) by ^{1}H NMR spectroscopy (Fig. 1). The aldehyde (H^a at 9.92 ppm) proton signal of **1** disappeared while a new peak (H^b at 4.98 ppm) appeared together with the upfield shifted aromatic protons (Fig. 1). The highly upfield shift of the aldehyde proton and the slight shifts of aromatic protons indicate a large electronic change that occurred in the aldehyde functional group rather than the aromatic group of **1**, plausibly to afford an aldol product (**2**). ^{13}C NMR and mass spectral analyses of **2** also showed corroborative evidences for the aldol reaction (see the Supplementary data).

Probe **1** has a UV–vis maximum centered at 449 nm ($\varepsilon = 4.8 \times 10^4\ M^{-1}\ cm^{-1}$) and displays relatively weak fluorescence. The addition of proline triggers a prominent hypsochromic shift of the absorption maximum to 378 nm ($\varepsilon = 2.3 \times 10^4\ M^{-1}\ cm^{-1}$) with



Scheme 1. Proline-catalyzed transformation of probe **1**–**2**.

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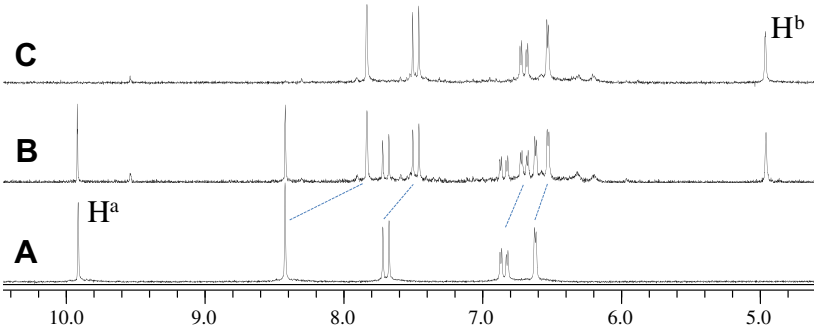

Figure 1. Partial ^{1}H NMR spectra of **1** (20 mM) upon the addition of L-Pro (0.5 equiv) in DMSO-d_6/acetone-d_6 (v/v, 4:1). (A) **1**, (B) **1** + Pro after 30 min, (C) **1** + Pro after 4 h.

a pseudo-isosbestic point at 403 nm. The reaction was almost complete within 6 h, whereas the blank reaction of **1** without L-Pro was so slow and its fluorescence intensity was negligibly changed even after 24 h (Fig. 2). The rate constant of the transformation in the presence of L-Pro was measured under a pseudo first-order condition to give $k_{obs} = 1.1 \times 10^{-4}$ s^{-1}. As expected, the probe (**1**) shows a strong blue fluorescence emission upon the addition of L-Pro, owing to the transformation of the carbonyl group into the alcohol product (**2**).[16] The fluorescence intensity of the probe was more than 100 times increased by L-Pro and reached the same intensity as that of **2** after 6 h (Fig. S2).

We wondered that other amino acids could mediate the aldol reaction of **1** or proline would be the only mediator for the aldol reaction. To evaluate the selectivity of **1**, we measured the fluorescence intensities for various amino acids with neutral, basic, and acidic side chains. The fluorescence intensity of **1** was enhanced more than 400 times by L-Pro with moderate selectivity in the absence of cobalt ions. When cobalt(II) ions were applied, the selectivity of **1** toward Pro was highly enhanced (Fig. 3). The

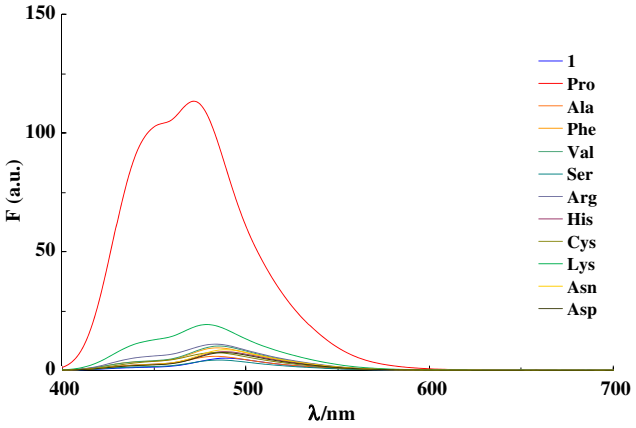

Figure 3. Fluorescence responses of **1** (20 μM in DMSO/acetone, 4:1) upon addition of amino acids (250 equiv) in the presence of cobalt(II) triflate (125 equiv). λ_{ex} = 380 nm.

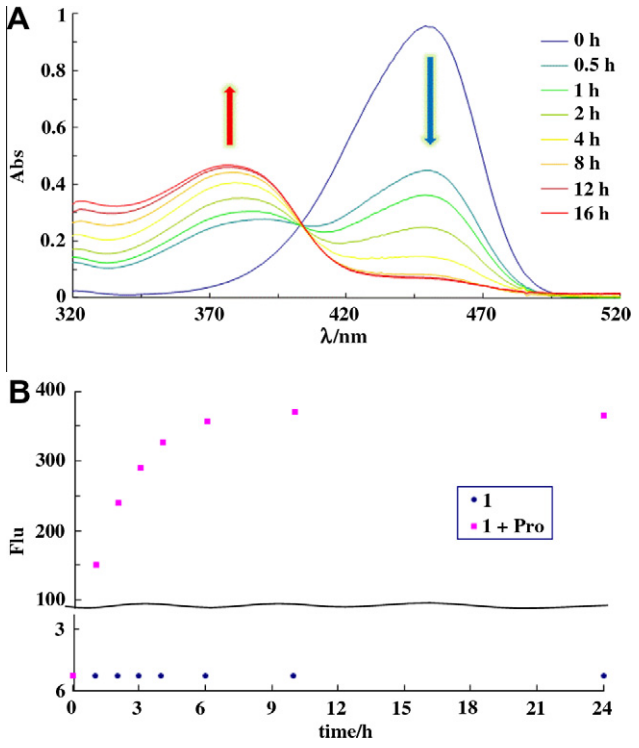

Figure 2. UV–vis spectral changes (A) and fluorescence kinetics (B, λ_{ex} 380 nm, λ_{em} 452 nm) upon the addition of 250 equiv L-Pro to **1** (20 μM) in DMSO/acetone (v/v, 4:1).

fluorescence intensity of **1** was more than 77 times increased by L-Pro, whereas the fluorescence enhancement (F/F_0) by other amino acids was less than 5 except lysine (F/F_0 = 9.7) (Fig. S3). This pronounced selectivity of **1** for L-Pro plausibly comes from the weak coordination of L-Pro to the cobaltous ion, therefore the better participation in the aldol catalysis compared to other amino acids having a primary amino group.

The prominent fluorescence changes of **1** were also observable by a hand-gun UV lamp (Fig. 4). Most amino acids including L-Pro did not change the solution color and remained as yellow as **1**, although some amino acids with a basic side chain (His, Arg, and Lys) displayed green colors. The bright blue fluorescence was only observed by L-Pro. The combined information of fluorescence and visible changes will be good to discriminate L-Pro from other amino acids.

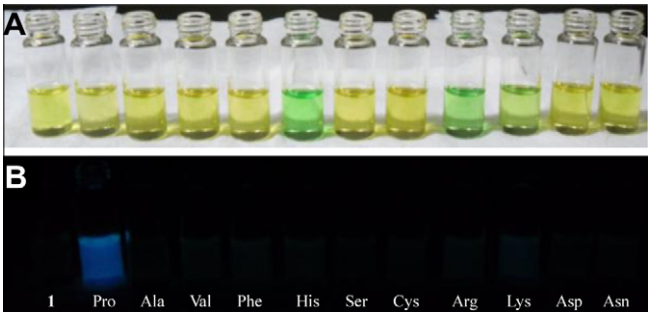

Figure 4. Naked-eye (A) and fluorescence (B) images of **1** (20 μM in DMSO/acetone, 4:1) upon addition of various amino acids (250 equiv) in the presence of cobalt(II) triflate (125 equiv). λ_{ex} = 365 nm.

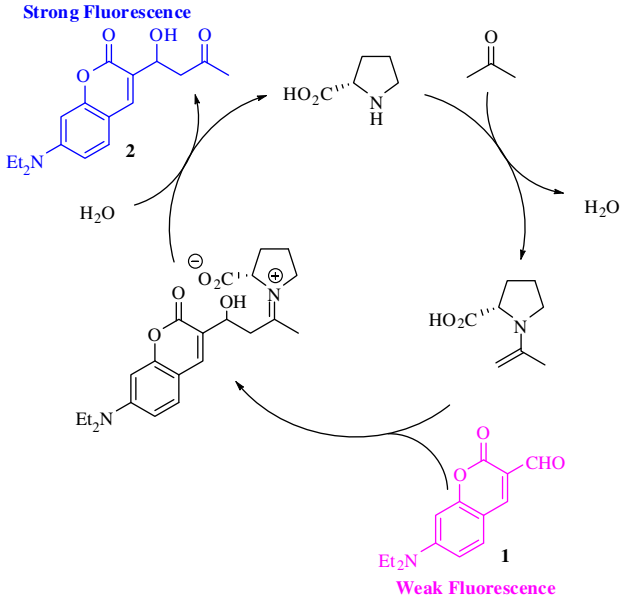

Figure 5. Proposed reaction mechanism.

To determine the limit of detection (LOD) of L-Pro by **1**, we measured the fluorescence intensities after the addition of L-Pro to **1** (20 μM). From the LOD measurement, we found that micromolar concentrations (1.2 μM) of L-Pro are detectable by **1** (Fig. S4).[17]

We propose an enamine-mediated aldol reaction for the selective and sensitive detection of proline (Fig. 5). The enamine formed from L-Pro and acetone is an active nucleophile and therefore attacks the electrophilic aldehyde (**1**). The intermediate, unstable iminium is easily hydrolyzable by a water molecule to afford an aldol product (**2**), while regenerating L-Pro as a catalyst. The aldol product (**2**) with an sp^3-hybrid carbon near the fluorophore can display relatively a strong fluorescence by reproducing the 'push–pull' electronic structure of coumarin.[5,18]

In conclusion, we prepared a coumarin-based aldehyde as an aldol reactant for the selective detection of proline by a proline-mediated aldol reaction. We found that probe **1** is a highly selective fluorescence turn-on probe for proline over other various amino acids with micromolar sensitivity. We expect that this probe should prove useful for the diagnosis of proline-related diseases. Further research is in progress.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.07.003.

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