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Fluorescein derivative-based, selective and sensitive chemosensor for NADH

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ABSTRACT

Fluorescein mercury acetate (FMA) derivative can be used as a chemosensor for NADH that plays key roles in cellular energy metabolism and dehydrogenase enzymatic reactions. The sensor recognizes NADH by two mercury metal ions in the compound using the metal–anion interaction and its subsequent binding-induced fluorescence changes of FMA. The FMA can detect an aqueous NADH concentration of approximately 1.0 μM and has a high selectivity over various anions including NAD^+, the oxidized form of NADH.

Nicotinamide adenine dinucleotide, NAD^+, and its reduced form, NADH, are ubiquitous biomolecules found in both eukaryotic and prokaryotic organisms and these molecules are the key central charge carriers in living cells.[1] The NADH/NAD^+ couple is known as coenzymes, the two nucleotides transfer hydrogen atoms and electrons from one metabolite to another in many cellular redox reactions. The couple is used as the cofactor of more than 300 dehydrogenase enzymatic reactions.[2] Specifically, NAD^+ and NADH play key roles in cellular energy metabolism such as ATP generation in mitochondria.[3] Therefore, it is important to evaluate the concentration of NADH for the understanding of overall cellular energy metabolism. Several methods are currently available for the determination of NADH, including enzyme-based method[4], chromatography[5], mass spectrometry[6], and the electrochemical method.[7] Although these methods are very sensitive and selective for NADH, they are not available for noninvasive monitoring of tissue energy metabolism. Therefore, the intrinsic fluorescence of NADH has been used as an extremely useful tool in the noninvasive monitoring of tissue energy metabolism.[8] However, the fluorescence of NADH is weak, 1/100 to 1/1000 the magnitude of conventional fluorophores and can be interfered by biomolecules in vivo because the fluorescence is near λ_{max} = 450 nm and the excitation wavelength is 340 nm.[8b] Recently, only limited fluorescent chemosensors satisfied with high quantum yield and a long emission wavelength have been developed.[9] Therefore, it is desirable to develop a fluorescent chemosensor for NADH with these properties.

Numerous sensors for anions, including phosphate derivatives, have been devised using electrostatic interactions, hydrogen bonding, and metal–ligand interactions for the recognition of analytes. A metal–ligand interaction is useful in the development of anion sensors because the metal–anion bonding interaction is very strong in water and the metal complexes that exhibit geometrical preference can selectively recognize target anions of a given shape.[10] Numerous metal complexes such as cyclene-Zn^{2+}, dipicolylamine-Zn^{2+}, and azamacrocyle-Cu^{2+} have been used in the design of anion sensors.[11] Specifically, metal complexes with two metal ion moieties as anion recognition sites are effective chemosensors of phosphate derivatives such as adenosine triphosphate (ATP), pyrophosphate, flavin adenine dinucleotide (FAD), and peptides with phosphotyrosine residues.[12] Additionally, it is well known that molecules bearing binding affinities to mercury can bind to two mercury ions in fluorescein mercury acetate and then the binding could be communicated as fluorescence changes.[13] With this information, we expected that FMA can be used as a fluorescent chemosensor for phosphate derivatives including NADH because FMA has two mercury ions as binding sites and the binding will induce fluorescent signals of the fluorescein. The FMA used in this study was synthesized from fluorescein and mercury acetate, as shown in Scheme 1.[14]

To evaluate the selectivity of FMA for various phosphate derivatives and anions including NADH, the fluorescence changes of FMA in the presence of these anions were measured. Fluorescence spectra of solutions of FMA (1 μM) recorded after the addition of 15 equiv of each anion are shown in Figure 1. FMA showed high selectivity for NADH compared to the other anions. No significant changes in the fluorescence intensity were observed upon the addition of any other anions. Specifically, NAD^+ and other phosphate anion derivatives, did not affect the fluorescence of FMA and only NADH induced a significant decrease of the fluorescence intensity. The data implied that the phosphate anion derivatives could bind to FMA but could not affect the fluorescence change

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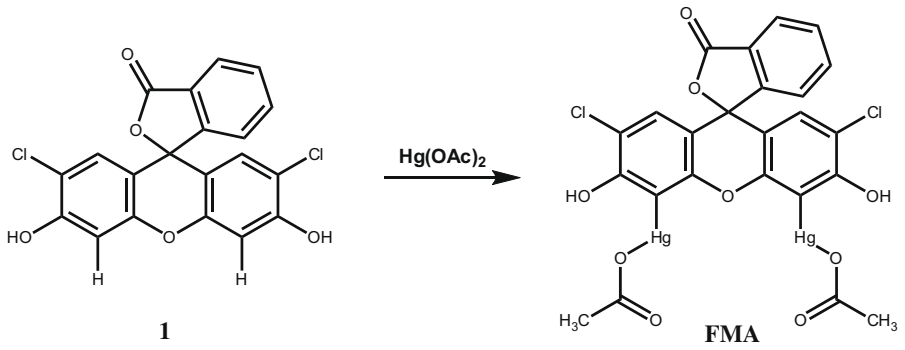


Scheme 1. Synthetic route to FMA.

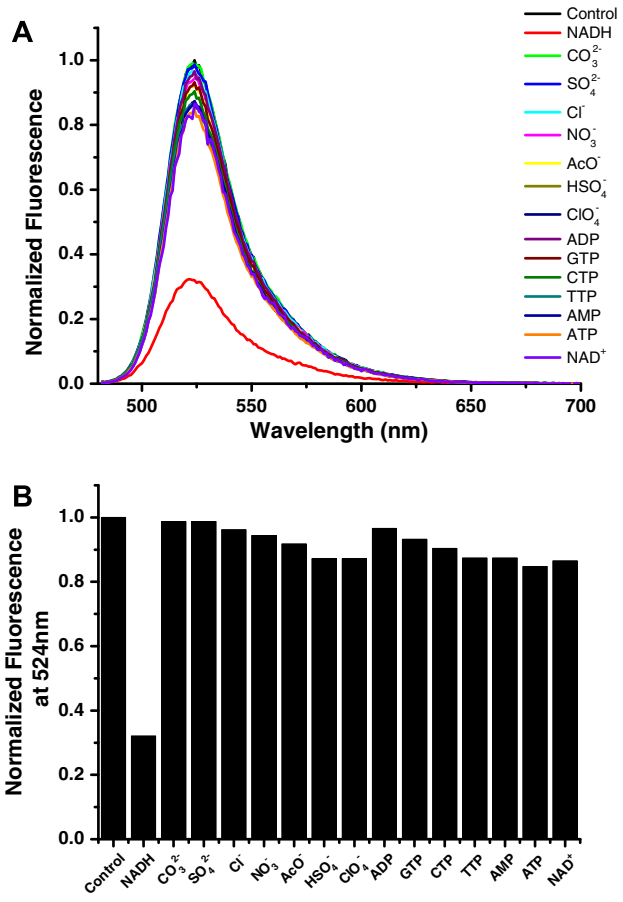


Figure 1. (A) Fluorescence emission spectra obtained by the addition of various anions to a pH 7.2 buffer solution containing FMA (1.0 μM). (B) Plot of normalized fluorescence intensities of FMA at 524 nm versus anions.

of FMA. Further, FMA was incubated with NADH in D_2O, and ^{31}P NMR spectrum of the solution was measured. As shown in Figure 2A, ^{31}P NMR signal of NADH was reduced with the addition of NADH and was saturated with 1 equiv of FMA.[15] The spectra showed that phosphate anion in NADH bound to FMA. The quenching of the fluorescence, presumably by an ET quenching route, was induced by the NADH cofactor which was substantially more efficient than the quenching of the FMA by the oxidized, NAD^+ cofactor.[9a] Alternatively, NADH is a reducing agent for donating electrons and, in some cases, the electron transfer reaction can change the structure of organic dyes.[16] Therefore, it is possible that the fluorescence of FMA in the presence of NADH can be changed by the electron transfer reaction. To eliminate the possibility of the fluorescence change of FMA by the deformation of structure of FMA via the electron transfer reaction, FMA (1 μM) was incubated with NADH (30 μM) for 4 h. The fluorescence of FMA with NADH was not changed for 4 h (see Supplementary data), which show that the fluorescence change of FMA in the presence of NADH was not due to the deformation of structure of FMA by the electron transfer reaction. In addition, a Job plot was performed to investigate the binding mode of NADH to FMA.[17] As shown in Figure 2, the major binding species was a 1:1 complex as depicted in Scheme 2, despite the 2:1 complex which may be included because Job plot is not a perfect symmetry.

Fluorescence emission spectra of FMA at pH 7.2 in the presence of varied concentrations of NADH are shown in Figure 3. The addition of NADH induced the decrease of fluorescence, and as shown in Figure 3B, the observed fluorescence intensity was nearly proportional to the NADH concentration. The binding affinities of NADH to FMA were 1.17×10^5 as determined by a non-linear regression analysis of the titration data.[16] Additionally, from the titration results, the detection limit of FMA for NADH was estimated as 1.2 μM (see Supplementary data). Unlike previous fluorescent chemosenors[9b,c], FMA is not a turn-on type chemosensor and such decreased emission is undesirable for analytical purposes. However, the sensor was highly sensitive for NADH and the fluorescence could not be interfered by biomolecules because the λ_{max} of FMA is a long wavelength unlike the intrinsic fluorescence of NADH.

Fluorescein derivatives are generally very sensitive to pH and have been used as a pH indicator. The effect of the pH value of the medium on NADH sensing of FMA was studied in the pH range of 6.0–9.0. Despite the pH-dependent fluorescence of FMA, the addition of NADH can induce large changes in the fluorescence. Although fluorescence intensity changes of FMA are slightly sensitive to pH, NADH can lead to a strong response in the pH range and the response is especially most distinct at a neutral pH. As a result, FMA could be used as a fluorescent chemosensor for NADH in a broad pH range (Fig. 4).

In conclusion, we synthesized FMA in a single step from commercially available dye and demonstrated that the compound is a highly effective fluorescent sensor for NADH. This sensor recognizes phosphates in NADH by two mercury metal ions using metal–anion interactions and the binding is induced as a fluorescent signal of FMA. This compound has a high sensitivity of 1.2 μM for the detection NADH. Additionally, this compound has a high selectivity for NADH over various anions and can discriminate between NADH and NAD^+. In addition, the sensor can be used for the assay of enzymes related to the NADH/NAD^+ couple reaction.

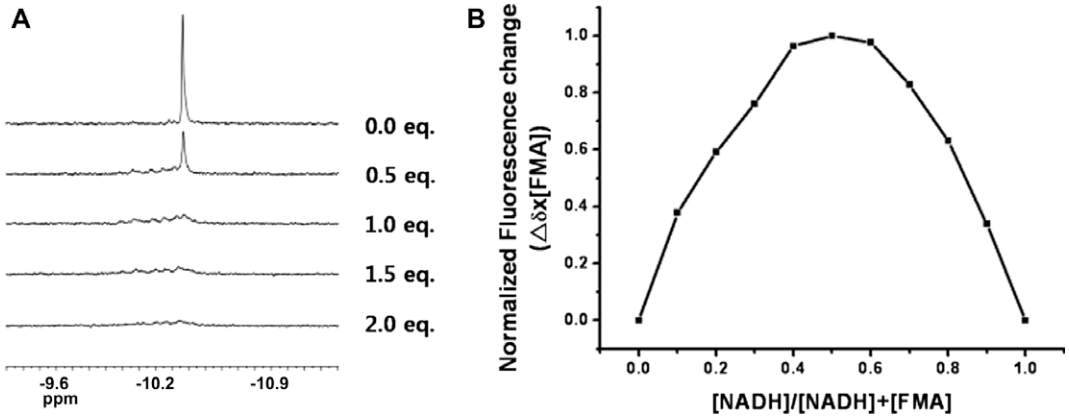
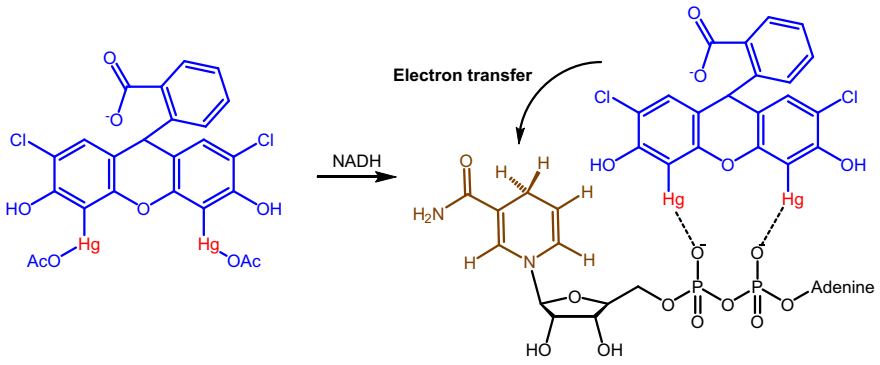


Figure 2. (A) ^{31}P NMR spectra of NADH (1.0 mM) in D_2O as additions of FMA with external reference (85% phosphoric acid). (B) The Job plot for binding of NADH with FMA in a pH 7.2 buffer solution. Aqueous solutions of FMA (5 μM) and NADH (5 μM) were mixed at varying ratios and the change in fluorescence at 524 nm was measured.



Scheme 2. Schematic illustration of the NADH sensor.

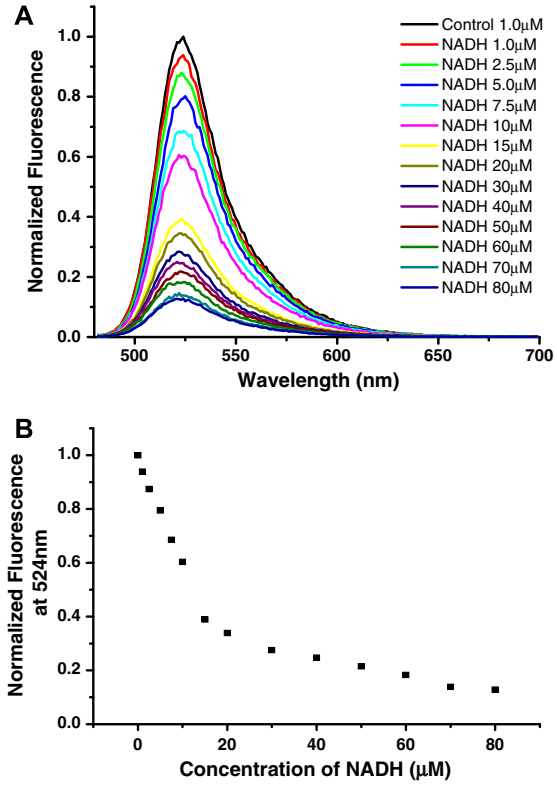


Figure 3. (A) Fluorescence emission spectra obtained by NADH addition (0–80 μM) to a pH 7.2 buffer solution containing FMA (1 μM). (B) Plot of normalized fluorescence intensities of FMA at 524 nm versus NADH concentration.

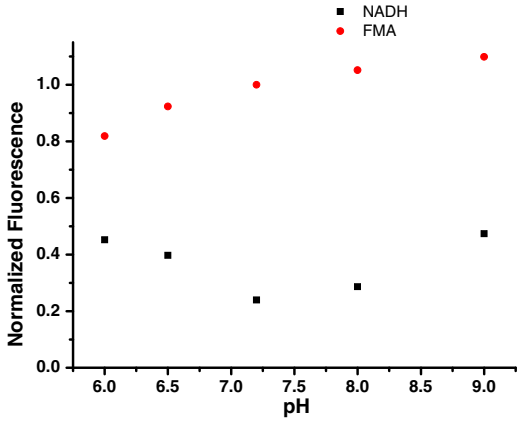


Figure 4. Fluorescence intensities of FMA (1 μM) at 524 nm in the presence and absence of NADH (30 μM) at each pH.

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Supplementary data

Supplementary data (experimental details of synthesis of FMA, the stability of FMA in the presence of NADH, determination of the detection limit of FMA for NADH) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.05.044.

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