ORGANIC LETTERS

2004
Vol. 6, No. 18
3107−3109

Three-Point Recognition and Selective Fluorescence Sensing of L-DOPA

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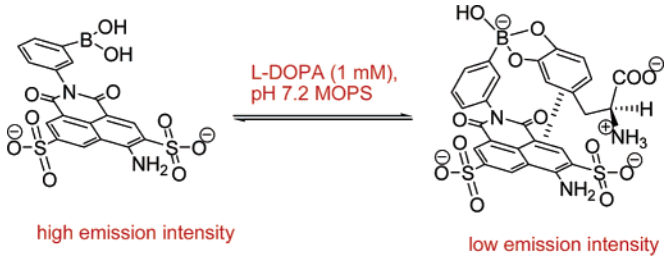
Received June 15, 2004

ABSTRACT



A phenylboronic acid derivative of a well-known dye (Lucifer yellow) recognizes L-DOPA through a combination of reversible esterification, charge transfer, and electrostatic interactions. The selective recognition event is signaled by a drop in the emission intensity of the fluorescent chemosensor.

The design and synthesis of effective fluorescent chemosensors for biologically relevant analytes is of paramount interest in supramolecular chemistry.[1] Catecholamines are involved in a number of biological processes, most of them directly related to significant health issues such as Parkinsonism, schizophrenia, and hypertension.[2] Dopamine is known to function as a brain and peripheral neurotransmitter in mammals,[3] and L-DOPA is the immediate precursor for the biosynthesis of dopamine in vivo. Considering the biochemical significance of catecholamines and their precursor L-DOPA, it is not surprising that a number of mechanisms operate in vivo for their homeostatic regulation.[4]

Following the pioneering works of Czarnik[5] and later Shinkai,[6] boronic acid derivatives have been used in the recognition and sensing of vicinal diols, carbohydrates, and catechols for the past 2 decades, with many successful examples emerging during this time period.[7] There have been reports of selective transport of catecholamines using arylboronic acids.[8] Earliest fluorescence sensing of L-DOPA was reported in 1993.[5b] In a more recent study,[9] Raymo presented a diazapyrene-based dopamine chemosensor attached to silica particles. Although there are alternative methods for determining catecholamine concentrations,[10] fluorescent chemosensors have the unique potential for real-time in vivo imaging of these analytes. It would suffice to consider the contributions made by fluorescent chemosensors of calcium to biological and medical sciences.[11]

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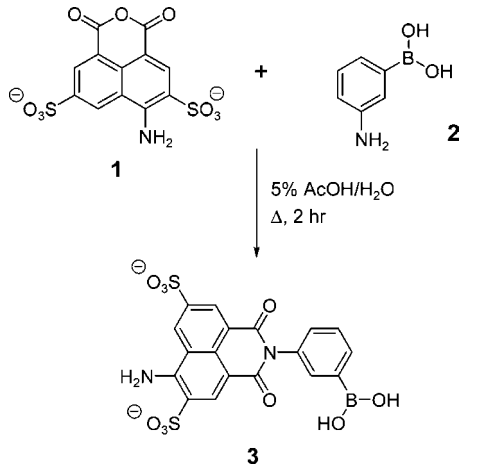
10.1021/ol0488744 CCC: $27.50
Published on Web 08/05/2004

In designing a fluorescent chemosensor for L-DOPA, we targeted dyes with complementary charged groups and electron-deficient π-systems. Recently, a number of reports appeared in the literature on the recognition of carbohydrates with naphthalenedicarboximide derivatives[12] at neutral pH. Thus, structurally related Lucifer yellow derivatives, with two sulfonate and two carbonyl functions on the naphthalene core, seemed to be an appropriate choice.

Scheme 1. Synthesis of Fluorescent Chemosensor **3**



Lucifer yellow dyes are water-soluble naphthalenedicarboximides that have found applications as polar tracers in neurobiology.[13] Their superior quantum yields and large Stokes' shifts together with excitability with various light sources (including blue laser diodes) and compatibility with fluorescein filter sets may have contributed to their popularity. In addition, the emission intensity of Lucifer dyes is pH-independent in a broad range of pH values. Although electrostatic interactions between the sensor and analyte would be highly diminished in a high dielectric constant solvent such as water, we expected that the additive nature of these interactions would still improve the selectivity and the binding constant. The target compound **3** was synthesized in analogy to the literature,[14] by reacting the commercially available anhydride (**1**) with 3-aminophenylboronic acid in aqueous acetic acid solution. The product was obtained in analytically pure state, following precipitation in the form of the dipotassium salt. Absorption spectrum in pH 7.2 buffer (MOPS, 0.1 M) displays a broad peak centered at 425 nm (extinction coefficient is 11,000 M^{-1} cm^{-1}), while the emission spectrum in optically dilute solutions displays a peak at 535 nm. Increasing concentrations of L-DOPA

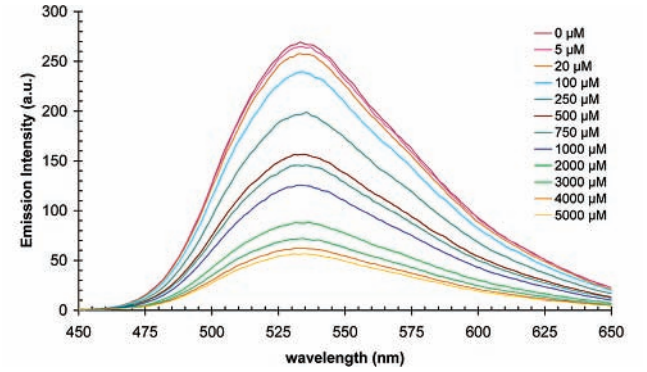


Figure 1. Emission spectrum of the chemosensor **3** (5 μM) in response to increasing concentrations of L-DOPA in buffered aqueous solutions (0.1 M MOPS) at pH 7.2. Excitation is at 430 nm.

quenches the fluorescence emission, decreasing the intensity to 1/5 of the emission intensity of the free chemosensor (Figure 1). To display the effectiveness of three-point recognition (namely, reversible boronic ester formation, $\pi-\pi$ interactions, and charge complementarity) of L-DOPA by the chemosensor **3**, we tested a set of structurally related compounds with incremental changes in functionalities. Thus, the set includes catechol, L-phenylalanine, L-tyrosine, and L- DOPA. The change in the emission intensity in response to increasing concentrations of these analytes is shown in Figure 2.

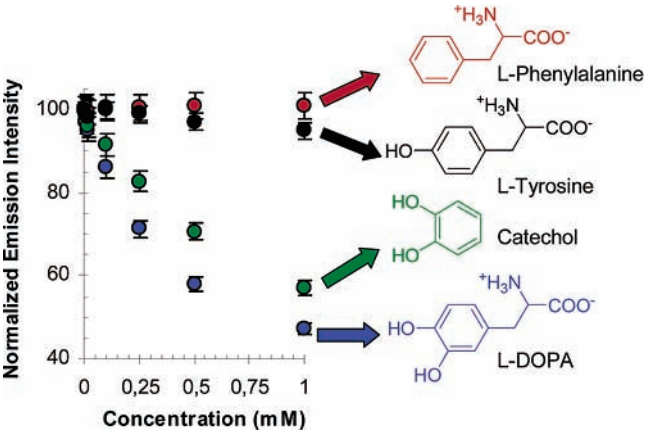


Figure 2. Normalized peak emission intensity of **3** in the presence of catechol, L-DOPA, and its natural precursors in buffered aqueous solutions (0.1 M MOPS, pH 7.2) Excitation wavelength is 430 nm. Error bars represent deviations from the average of 5 different runs.

As predicted, maximum change in the signal intensity was observed with L-DOPA, followed by catechol. Benesi–Hildebrand analysis of the emission data yielded a dissociation constant, K_d, of 6.1 $\times$ 10^{-4} (M) for 1:1 interaction. L-Phenylalanine did not cause any change in the emission

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intensity, and L-tyrosine at 1 mM concentration caused only a 5% decrease in the emission intensity.

Although the trend observed in the emission changes strongly suggest the participation of multiple interactions, we wanted to demonstrate the boronic acid−aromatic diol interaction explicitly. Thus, an NMR titration of 1 mM of the chemosensor **3** in buffered D_2O with L-DOPA was carried out (Figure 3). On increasing the concentration of L-DOPA,

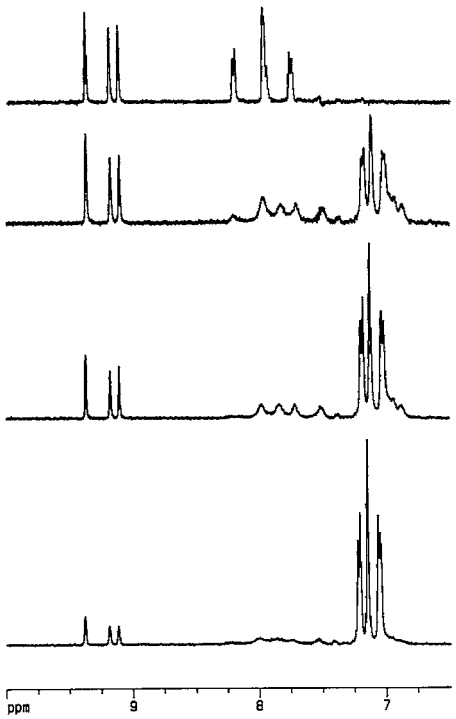

Figure 3. ^{1}H NMR titration of compound **3** (1 mM) with increasing concentrations (top to bottom) of L-DOPA in buffered D_2O solutions at pH 7.2 (0.1 M phosphate). L-DOPA concentration is (from top to bottom) 0, 2.5, 5.0, and 10 mM.

only the aromatic protons of phenylboronic acid moiety are affected. Reversible esterification, although broadening these peaks, induces an upfield shift of 0.25 ppm. The energy minimized structure (MM+, Hyperchem, v.7.5) of the complex places the L-DOPA and the naphthalenediimide π-systems at close proximity for charge transfer interactions and electrostatic attraction between the oppositely charged sulfonate and ammonium functionalities.

To experimentally verify the relevance of charge complementarity, we studied L-DOPA binding to the chemosensor **3** in the presence of buffered aqueous solutions of varying ionic strengths (Figure 4). Although it is known that ionic strength of a solution alters the emission characteristics of fluorophores in general, we observed that the direction of

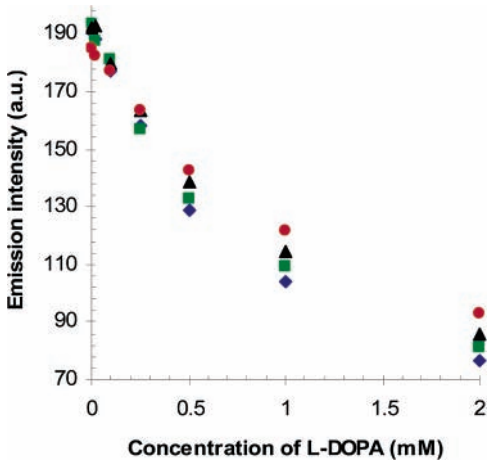

Figure 4. Effect of ionic strength on binding interactions. L-DOPA titration of chemosensor **3** (5 μM) in the presence of varying concentrations of $NaClO_4$: (blue diamonds) 0 M; (green squares) 0.25 M; (blue triangles) 0.50 M, (red circles) 1.0 M. Solutions were buffered with 0.1 M MOPS at pH 7.2. Excitation wavelength is 430 nm.

emission difference is reversed when L-DOPA is added. Thus, we clearly demonstrated that the lesser decrease in the emission intensity in the presence of L-DOPA in higher ionic strength solutions is due to weaker binding. Benesi−Hildebrand analysis also demonstrates that the dissociation constant increases to 9.5×10^{-4} M in the presence of 1.0 M $NaClO_4$. On the other hand, carbohydrates tested (glucose, fructose, mannose, and galactose) did not cause any changes in the emission spectrum at 5 mM concentration. A very recent literature report[15] (published during the preparation of this manuscript) about carbohydrate sensing with a number of boronic acid derivatives including **3** also confirms our findings; apparently the emission spectrum of **3** is not altered by carbohydrates even at much higher concentrations (near 100 mM).

Thus, we demonstrated that compound **3** is a selective chemosensor for L-DOPA under physiological conditions. Further structural modifications incorporating additional recognition elements are likely to produce a practically useful, selective imaging agent for L-DOPA and catecholamines.

Acknowledgment. This work was supported in part by a grant from DPT (BAP-01-03-DPT2003K120920-07) and the Turkish Academy of Sciences (TUBA). A.C. thanks the Scientific and Technical Research Council of Turkey (TUBITAK) for a scholarship.

OL0488744

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