ORGANIC LETTERS

2008
Vol. 10, No. 23
5369−5372

Synthesis of a New Water-Soluble Oligo(phenylenevinylene) Containing a Tyrosine Moiety for Tyrosinase Activity Detection

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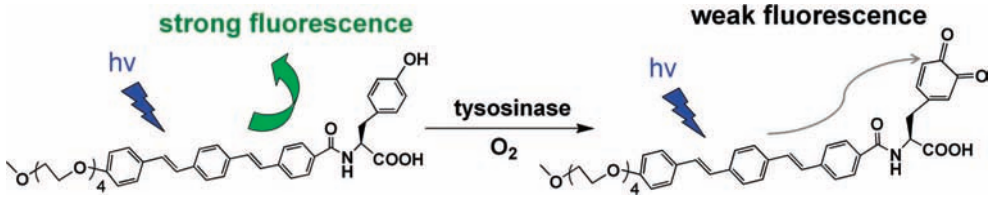
Received September 22, 2008

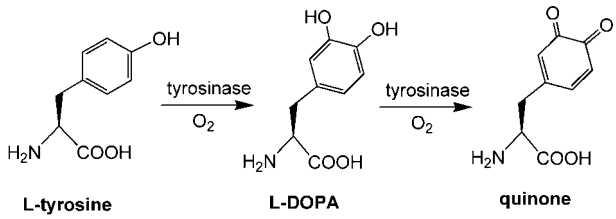
ABSTRACT



A new water-soluble oligo(phenylenevinylene) containing a tyrosine unit (OPV-Tyr) was synthesized as a fluorescent probe to optically detect tyrosinase activity. Upon the addition of tyrosinase, the tyrosine moiety was oxidized to quinone, which quenched the fluorescence of OPV-Tyr via intramolecular electron transfer from the phenylenevinylene unit to the quinone site. OPV-Tyr was elaborated to detect tyrosinase activity both in aqueous buffer solution and in agarose gel.

Tyrosinase is a copper-containing protein that can catalyze the oxidation of tyrosine to L-DOPA followed by subsequent oxidation to quinone (Scheme 1).[1] Accumulated tyrosinase is considered to be a marker for melanoma cancer cells,[2] and it is also a very important enzyme in the food industry.[3] The traditional assay method for tyrosinase based on colorimetry has an obvious drawback of limited sensitivity, although it is direct and quantitative.[4] Recently electrochemical and spectrophotometric methods have been developed by the Willner group for tyrosinase analysis using functionalized nanoparticles.[5] Although fluorescence technique is one of the most useful analytical tools in bioanalysis, only semiconductor quantum dots have been reported as fluorescent probes for tyrosinase analysis.[6]

Scheme 1. Oxidation of Tyrosine to Quinone by Tyrosinase

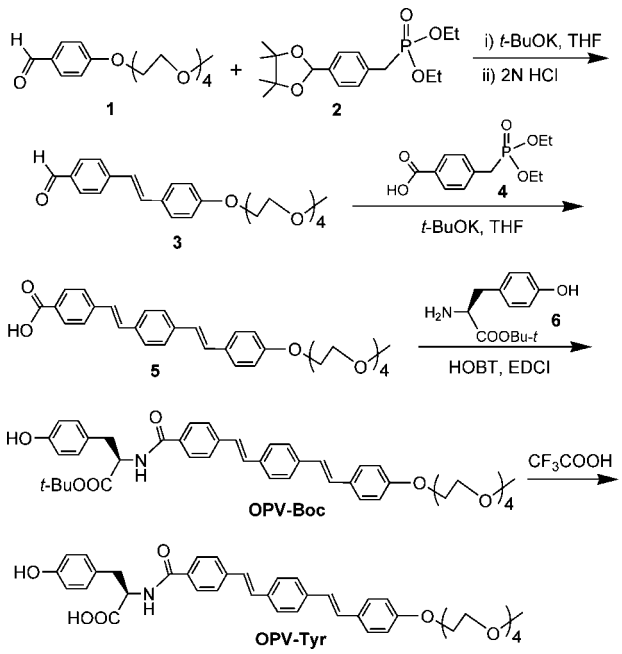


(1) Yildiz, H. B.; Freeman, R.; Gill, R.; Willner, I. *Anal. Chem.* **2008**, *80*, 2811.
(2) Angeletti, C.; Khomitch, V.; Halaban, R.; Rimm, D. L. *Diagn. Cytopathol.* **2004**, *31*, 33.
(3) (a) Mayer, A. M. *Phytochemistry* **1987**, *26*, 11. (b) Friedman, M. *J. Agric. Food Chem.* **1996**, *44*, 631.
(4) Gauillard, F.; Richard-Forget, F.; Nicolas, J. *Anal. Biochem.* **1993**, *215*, 59.
(5) (a) Li, D.; Gill, R.; Freeman, R.; Willner, I. *Chem. Commun.* **2006**, 5027. (b) Baron, R.; Zayats, M.; Willner, I. *Anal. Chem.* **2005**, *77*, 1566. (c) Freeman, R.; Elbaz, J.; Gill, R.; Zayats, M.; Willner, I. *Chem. Eur. J.* **2007**, *13*, 7288.
(6) Gill, R.; Freeman, R.; Xu, J. P.; Willner, I.; Winograd, S.; Shweky, I.; Banin, U. *J. Am. Chem. Soc.* **2006**, *128*, 15376.

10.1021/ol802210s CCC: $40.75
Published on Web 11/01/2008

Significant interest has recently been directed toward the synthesis of new oligo(phenylenevinylene) derivatives because of their well-defined molecular structures and excellent optoelectronic properties. They are widely used in optoelectronic devices, such as light-emitting diodes and nonlinear optical devices.[7] Herein, we report the synthesis of a new water-soluble oligo(phenylenevinylene) containing tyrosine moiety (OPV-Tyr) as a fluorescent probe to optically detect tyrosinase activity. Solubility in water is essential for interfacing with biomacromolecular enzymes, and this property was achieved by attaching polyethylene glycol pendent group to the oligo(phenylenevinylene) backbone. The OPV-Tyr itself emitted strong fluorescence. Because the quinone was an efficient electron quencher,[6] upon additions of tyrosinase, the tyrosine moiety was oxidated into quinone, and the OPV-Tyr demonstrated intramolecular electron transfer from phenylenevinylene units to the quinone sites, followed by the fluorescence quenching of OPV-Tyr. By triggering the change of emission intensity of OPV-Tyr, it was possible to assay tyrosinase activity.

Scheme 2. Synthesis of OPV-Tyr



Scheme 2 depicts the synthesis of OPV-Tyr. Reaction of **1**[8] with **2**[9] in the presence of *t*-BuOK in THF following treatment with 2 N HCl provided **3** in 75% yield. The compound **5** was obtained by reaction of **3** with **4** in the presence of *t*-BuOK in THF in 62% yield. The synthetic approaches for **3** and **5** are designed on the basis of the Wittig−Horner reaction.[10] After reaction of **5** with **6** in the presence of carbodiimides (EDCI) and 4-carboxybenzenesulfonamide (HOBT) in unhydrous DMF, the Boc-protected OPV-Boc was obtained in 57% yield. The Boc group of OPV-Boc was removed by trifluoroacetic acid (TFA) to get target OPV-Tyr in 99% yield. The ^{1}H NMR spectroscopies of OPV-Boc and OPV-Tyr are given in Figure S1 in Supporting Information. We could find that the proton peaks of Boc (δ_{CH_3} = 1.45 ppm) group in OPV-Boc disappeared and a new peak at 12.69 ppm (-COOH) appeared. These results indicated that the protected Boc groups were removed entirely upon treatment with TFA.

The OPV-Tyr was readily dissolved in polar solvents, such as MeOH, DMSO, DMF, and water. Its absorption and fluorescence spectra were measured in pure water solution (Figure 1). It exhibits an absorption maximum at 330 nm,

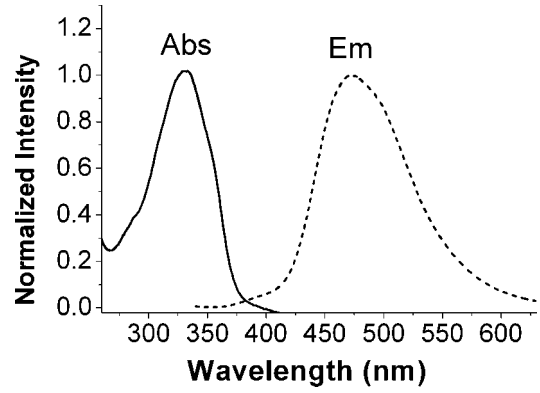


Figure 1. Absorption and emission spectra of OPV-Tyr in pure water solution. [OPV-Tyr] = 5.0×10^{-6} M.

which corresponds to the $\pi-\pi^*$ transition of the conjugated backbone. The OPV-Tyr emits bright blue-green fluorescence in water and shows emission maximum at 467 nm with the excitation wavelength at 330 nm. The fluorescence quantum yield of OPV-Tyr was calculated to be 42% in aqueous solution with quinine sulfate in water as the standard.[11]

Figure 2a shows the fluorescence spectral changes of OPV-Tyr as a function of the incubating time of tyrosinase. In these experiments, to a solution of OPV-Tyr ([OPV-Tyr] = 5.0×10^{-6} M) in 1 mL of phosphate buffer (50 mM, pH 7.4) was added tyrosinase ([tyrosinase] = 0.05 units mL^{-1}) at 28 °C, and the emission spectra were measured at 2 min intervals over 38 min with an excitation wavelength of 330 nm. Figure 2b shows the emission intensity of OPV-Tyr and **5** at 467 nm as a function of the tyrosinase incubating time.

(7) (a) Tang, C. W.; Van Slyke, S. A.; Chen, C. H. *J. Appl. Phys.* **1989**, *65*, 3610. (b) Service, R. F. *Science* **1996**, *273*, 878. (c) Sheats, J. R.; Antoniadis, H.; Mueschen, M.; Leonard, W.; Miller, J.; Moon, R.; Roitman, D.; Stocking, A. *Science* **1996**, *273*, 884. (d) Bosshard, C.; Sutter, K.; Pretre, P.; Hulliger, J.; Florsheimer, M.; Kaatz, P.; Gunter, P. *Organic Nonlinear Optical Materials*; Gordon and Breach Science Publishers: Amsterdam, The Netherlands, 1995; Vol. 1.

(8) Lottner, C.; Bart, K. C.; Bernhardt, G.; Brunner, H. *J. Med. Chem.* **2002**, *45*, 2079.

(9) Nierengarten, J.-F.; Eckert, J.-F.; Nicoud, J.-F.; Ouali, L.; Krasnikov, V.; Hadziioannou, G. *Chem. Commun.* **1999**, 617. Rich, D. H.; Gurwara, S. K. *J. Am. Chem. Soc.* **1975**, *96*, 1275.

(10) Ichimura, K.; Komatsu, T. *J. Polym. Sci. Polym. Chem.* **1987**, *25*, 3063.

(11) Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*; Springer: New York, 1999.

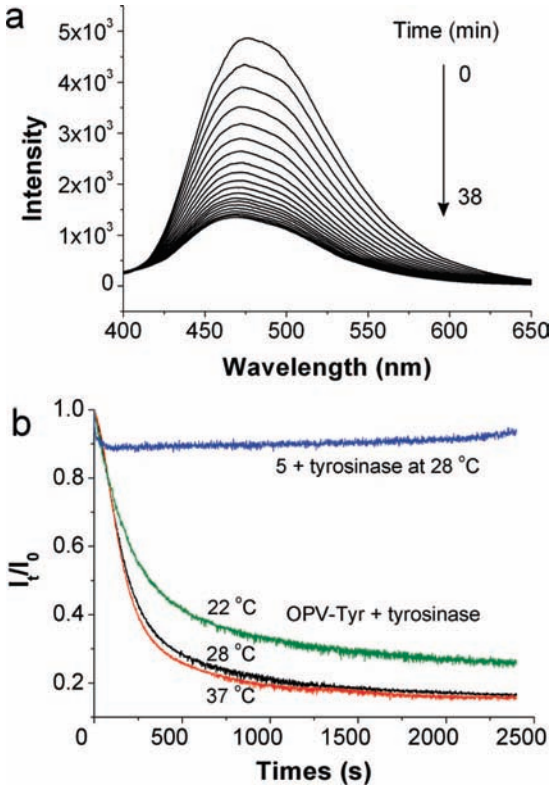


Figure 2. (a) Emission spectra of OPV-Tyr in as a function of the incubating time of tyrosinase at 28 °C. (b) Emission intensity of OPV and **5** at 467 nm as a function of the incubating time of tyrosinase at different temperatures. The measurements were performed in phosphate buffer solution (50 mM, pH 7.4). [OPV] = [**5**] = 5.0×10^{-6} M, [tyrosinase] = 0.05 units mL^{-1}. The excitation wavelength is 330 nm.

The initial solution of OPV-Tyr emitted strong fluorescence. After adding tyrosinase, the emission of OPV-Tyr was gradually quenched with the incubating time from 0 to 38 min and reached the plateau after 32 min, which indicated that the oxidation of tyrosine moiety was nearly completed. As shown in Figure 2b, the increase in the incubation temperature gave rise to a fast enzymatic reaction rate and a higher level of fluorescence quenching of OPV-Tyr. The enzymatic process was verified by UV spectra which is a well-known method for tyrosinase activity assay. As shown in Figure S2 in Supporting Information, a new peak with maximum at 440 nm appeared, which provided evidence that indeed oxidation from tyrosine to quinone took place.[4] Control experiment showed that tyrosinase has little effect on fluorescence signal of **5** without tyrosine moiety (Figure 2b). These observations indicated that the fluorescence quenching of OPV-Tyr originated from the formation of quinone, and the OPV-Tyr can be utilized as a probe to assay the tyrosinase in real time.

We also studied the activity of tyrosinase in the presence of inhibitor. Figure 3 showed the changes of maximum emission intensity of OPV-Tyr at 467 nm in the presence of

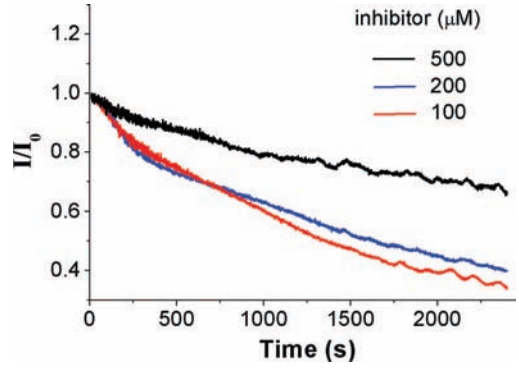


Figure 3. Emission intensity of OPV-Tyr at 467 nm as a function of the tyrosinase incubating time with varying concentrations of inhibitor. The measurements were performed in phosphate buffer solution (50 mM, pH 7.4). [OPV-Tyr] = 5.0×10^{-6} M, [tyrosinase] = 0.05 units mL^{-1}, [inhibitor] = 100−500 μM. The molar ratio of inhibitor to enzyme is in the range of 1.2−6.2. The excitation wavelength is 330 nm.

benzaldehyde, a common inhibitor of tyrosinase.[12] Increasing the inhibitor concentration from 100 to 200 μM exhibited limited effect on the activity of the enzyme. However, when the concentration of the inhibitor was increased to 500 μM, it was observed that the quenching process of OPV-Tyr can be inhibited signifcantly, which indicated the tyrosinase activity was inhibited. These data indicate that OPV-Tyr could be used as a probe to assay tyrosinase activity, as well as to screen potential inhibitors.

Hydrogels with interstitial spaces filled with water have received significant attention because of their broad applications in food, medicines, biomaterials, cosmetics, separation technology, etc.[13] Among them, agarose gel is one of the most widely used hydrogels, especially in biomedical research, because it provides the mimic environment of the cell. Hence, we examined the above enzyme responsive behavior in agarose gel. In these experiments, the agarose gel gelating the phosphate buffer solution (50 mM, pH 7.4) containing OPV-Tyr (5.0×10^{-6} M) was first prepared, then tyrosinase ([tyrosinase] = 0.05 units mL^{-1}) was added above the gel followed by incubation at 30 °C for specific times. The gel doped with OPV-Tyr emitted strong blue-green fluorescence. After 50 min, the fluorescence of the gel initial agarose was obviously quenched. About 150 min later, the fluorescence was fully quenched (Figure 4), which indicated that the oxidation of tyrosine moiety was nearly finished in agarose gel. In comparison with that in solution, the enzyme reaction in hydrogel is slower. Since the environment of the agarose gel is more similar to the cytosol of the cell, it paves the way for the further detection of tyrosinase activity using OPV-Tyr as a fluorescence probe in cells.

(12) Jiménez, M.; Chazarra, S.; Escribano, J.; Cabanes, J.; Garcia-Carmona, F. *J. Agric. Food Chem.* **2001**, *49*, 4060.
(13) (a) *Polymer Gels: Fundamentals and Biomedical Applications*; Rossi, D. De., Kajiwara, K., Osada, Y., Yamauchi, A., Eds.; Plenum Press: New York, 1991. (b) *Hydrogels in Medicine and Pharmacy*; Peppas, N. A., Ed.; CRC Press: Boca Raton, 1987; *Vol. III: Properties and Applications*. (c) Silva, G. A.; Czeisler, C.; Niece, K. L.; Beniash, E.; Harrington, D.; Kessler, J. A.; Stupp, S. I. *Science* **2004**, *303*, 1352.

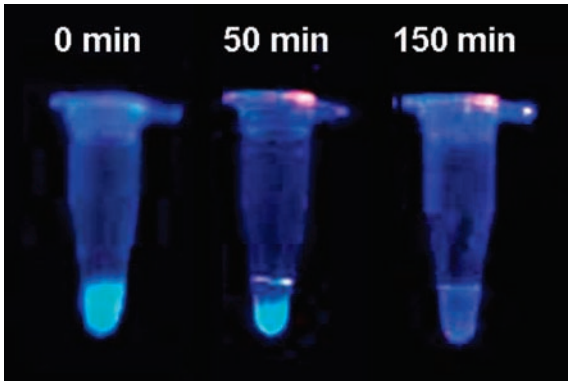


Figure 4. Photographs of tyrosinase activity detection in agarose gel under UV light. [tyrosinase] = 0.05 units mL^{-1}.

In summary, a new water-soluble oligo(phenylenevinylene) containing tyrosine moiety (OPV-Tyr) was synthesized and characterized. Upon additions of tyrosinase, the tyrosine moiety was oxidated into quinone with quenching ability, and the OPV-Tyr demonstrated intramolecular electron transfer from phenylenevinylene unit to the quinone site, followed by the fluorescence quenching of OPV-Tyr. The inhibitor of tyrosinase blocked the catalytic behavior. The OPV-Tyr can act as a fluorescent probe to optically detect tyrosinase activity, as well as to screen potential inhibitors of tyrosinase. Except for in aqueous buffer solution, the tyrosinase activity can also be detected in agarose gel which mimics the environment of the cell. The OPV-Tyr shows the potential for the detection of tyrosinase activity in cells.

Acknowledgment. The authors are grateful for the financial supports from the "100 Talents" program of Chinese Academy of Sciences, the National Natural Science Foundation of China (nos. 20725308, 20721061, and 20574073), the National Basic Research Program of China (no. 2006CB806200), and the Major Research Plan of China (no. 2006CB932100).

Supporting Information Available: Experimental details for synthetic procedures and enzyme analysis. ^1H NMR spectra of OPV-Boc in $CDCl_3$ and OPV-Tyr in DMSO-d_6. Absorption spectra of OPV-Tyr before and after addition of tyrosine. This material is available free of charge via the Internet at http://pubs.acs.org.

OL802210S