Poly(3,4-Ethylenedioxythiophene) Derivatives with Electrochemical Chiral Sensor for 3,4-Dihydroxyphenylalanine Discrimination

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***Abstract*—Two chiral poly(3,4-ethylenedioxythiophene) (PEDOT) derivatives, poly(N-(tert-butoxycarbonyl)-L-phenylalayl (3,4-ethylenedioxythiophene-2'-yl)methylamide) (PEDOT-Boc-Leu) and poly(L-phenylalayl (3,4-ethylenedioxythiophene-2'-yl)methylamide) (PEDOT-Leu) modified electrodes were used to recognize 3,4-dihydroxyphenylalanine (DOPA) enantiomers by square wave voltammetry (SWV) and cyclic voltammetry (CV) in sulphuric acid solution. It was amazing that the SWV peak currents of enantiomers were found to be quite different and hence the enantiomers could be successfully recognized. However, no obvious difference was observed by the method of CV. Satisfactory results implied that the obtained polymer films could play crucial roles in the development of practical value and analytical application prospects.**

Keywords-chiral poly(3,4-ethylenedioxythiophene); dihydroxyphenylalanine enantiomers; recognize; square wave voltammetry

I. INTRODUCTION

3, 4-Dihydroxyphenylalanine (DOPA) is an important neurotransmitter biosynthesized naturally in humans by tyrosinasecatalyzed oxidation of tyrosine in a melanin biosynthetic pathway, and by tyrosine hydroxylase in brain for catecholamine neurotransmitter biosynthesis. It also plays an important role in adhesive and cross-linking property of mussel adhesive proteins [1].On the other hand, DOPA has also been incorporated into proteins by in vitro protein synthesis and solid-phase peptide synthesis [2]. However, DOPA has L- and D- two kinds of configurations due to the particularity of DOPA structure, and the difference for the performance of different configuration is very big. L-DOPA has been widely used in the treatment of Parkinson's disease for more than forty years and it plays a very crucial role in clinic and neurochemistry [3-5]. On the contrary, D-DOPA is inactive and has toxic properties [6, 7]. Owing to different metabolisms of the active and inactive components, using racemic mixtures containing D- and L-DOPA may cause serious side effects [8, 9]. Therefore, the study of chiral recognition of DOPA enantiomers is very necessary. Many methods have been reported to identify enantiomers. These included high-performance liquid chromatography (HPLC) [10-13], capillary electrophoresis (CE) [14-17], electrokinetic chromatography (EKC) [18-19], and microchip electrophoresis (MCE) [20, 21]. Most of these analytical methods deployed ultraviolet (UV) detection that lacked sensitivity and the capability of peak identification. In comparison with these methods, electrochemical methods, which have advantages of low cost, high speed and high sensitivity, are always regarded as the lowest cost effective for the discrimination of DOPA.

poly(3,4-ethylenedioxythiophene) (PEDOT) is one of representatives and highly promising conducting polymers with versatile properties such as high electrical conductivity, low band gap, good redox activity, thermal stability, long-term stability, and excellent transparency in the doped state [22]. Also PEDOT is applied in a variety of areas, such as electrolytic capacitors [23], ITO substitution [24], antistatic coatings [25, 26], organic solar cells [27,28], electrochromics [29-31], and so on. Chiral PEDOT is a member of PEDOT family, which has attracted considerably widespread interest due to its fascinating properties. Importantly, to the best of our knowledge, the study of using the property of chiral PEDOT for the recognition of chiral drugs still remains blank.

In this work, two PEDOT derivatives, poly(*N*-(tert-butoxycarbonyl)-L-leucyl(3,4-ethylenedioxythiophene-2′-yl) methylamide) (PEDOT–Boc–Leu) and poly(L-leucyl(3,4-ethylenedioxythiophene-2′-yl)methylamide) (PEDOT–Leu), were electropolymerized (Scheme 1). A simple and fast procedure was used for the fabrication of electrodes with chiral PEDOT–Boc–Leu and PEDOT–Leu. The obtained electrodes were used to identify of DOPA enantiomers by methods of cyclic voltammetry (CV) and square wave voltammetry (SWV). The peak currents for the enantiomers were found to be different from each other when D- and L-DOPA were detected by square wave voltammetry (SWV). Hence, the combination of SWV with chiral PEDOT–Boc–Leu and PEDOT–Leu showed the chiral discrimination ability for DOPA.

II. EXPERIMENTAL

A. Materials

Enantiomerically pure 3,4-dihydroxyphenylalanine (DOPA, 99%), L-Leucine (99%), trifluoroacetic acid (TFA, 99%), and sulfuric acid (H_2SO_4, 98%) were purchased from Aladdin Chemistry Co. Ltd. Dichloromethane (CH_2Cl_2, AR; Tianjin Damao Chemical Reagent Factory) was purified by distillation over calcium hydride before used. Tetra-n-butylammonium hexafluorophosphate (Bu_4NPF_6, 99%; Acros Organics) was dried under vacuum at 60 °C for 24 h before used. *N*-(tert-butoxycarbonyl)-L-leucyl(3,4-ethylenedioxythiophene-2′-yl)-methylamide (EDOT–Boc–Leu) and L-leucyl(3,4-ethylenedioxythiophene-2′-yl)methylamide (EDOT–Leu) were prepared in accordance with Duan et al. [32].

B. Fabrication of sensor

The glassy carbon electrode (GCE) with a diameter of 3 mm served as the working electrode, and two Pt wires with a diameter of 0.5 mm were used as the counter electrode and the reference electrode. The counter electrode was carefully polished with abrasive paper (1500 mesh). The GCE was polished with alumina (Al_2O_3, 0.05 μm). Then, the counter electrode and the GCE was ultrasonically cleaned in turn with deionized distilled water, ethanol, and deionized distilled water each for 5 min, respectively. Then, they were dried in air before the experiment. Three electrodes in the cell were placed 5 mm apart during electrochemical measurements. To fabricate a sensor, a GCE was used as the working electrode, a platinum wire as an auxiliary electrode and a saturated calomel reference electrode (SCE). The PEDOT–Boc–Leu film was performed by the chronoamperometry (I-t) method at 1.6 V prepared in CH_2Cl_2–Bu_4NPF_6 (0.10 M) containing 0.01 M EDOT–Boc–Leu at room temperature in a one-compartment three-electrode cell. And the PEDOT-Leu film was performed by the I-t method at 0.75 V in CH_2Cl_2–Bu_4NPF_6 (0.10 M) containing 5% TFA and 0.01 M EDOT–Leu in a one-compartment three-electrode cell. The obtained PEDOT–Boc–Leu and PEDOT-Leu modified GCE were washed repeatedly with CH_2Cl_2 to remove the electrolyte and monomer, and dried in air.

III. RESULTS AND DISCUSSION

A. Application of chiral discrimination

The electrochemical behaviours of the enantiomeric pairs (D- and L-DOPA) were studied by CV and SWV, respectively. There is no apparent Faradic response of DOPA at the bare glassy carbon electrode (GCE) in 0.25 mol L^{-1} H_2SO_4 containing 0.5μmol L^{-1} L-DOPA (dashed line) or D-DOPA (solid line). This result might due to the concentration of samples too low to be detected for the bare GCE. As shown in Figure 1A, when the GCE was modified by chiral PEDOT-Boc-Leu, a pairs of significant redox peaks were observed in the CV curves owing to two-electron-two-proton oxidation and reduction of DOPA/dopa quinone in this system [9].

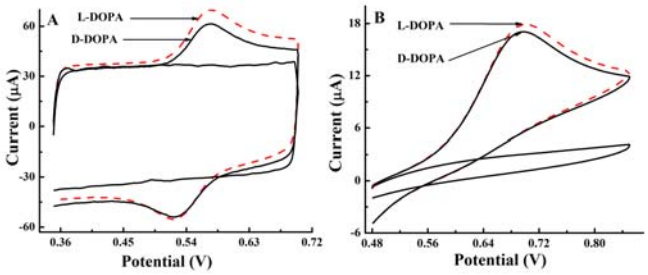

Figure 1. Cyclic voltammograms of PEDOT-Boc-Leu (A) and PEDOT-Leu (B) modified electrodes in 0.25 mol L^{-1} H_2SO_4 containing 0.5μmol L^{-1} L-DOPA (dashed line) or D-DOPA (solid line). Cyclic voltammogram: scan rate, 50 mV s^{-1}.

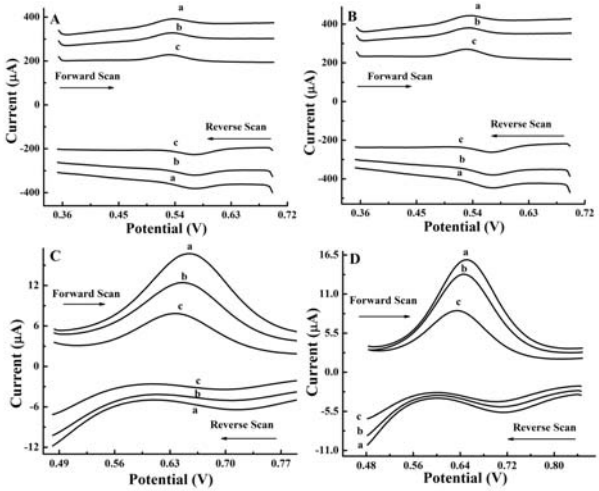

Figure 2. Square wave voltammetrys of PEDOT-Boc-Leu (A and B) and PEDOT-Leu (C and D) modified electrodes in 0.25 mol L^{-1} H_2SO_4 containing 0.5μmol L^{-1} L-DOPA (A and C) or D-DOPA (B and D). Square wave voltammetry: step height, 0.004 V; frequency, 5Hz (c), 10Hz (b), 15 Hz (a).

Also, the modified of chiral PEDOT-Leu only showed one peak (Figure 1B), and this result might attribute to the amino of PEDOT-Leu. Thus D- and L-DOPA cannot be recognized with the CV technique in the same system. It was presented that the sensitivity of PEDOT-Boc-Leu and PEDOT-Leu modified GCEs is better than bare GCE in the same situation. Theoretically, more modification favours the enantioselective effect. But they may decrease the electrochemical response and had a bad effect on the sensor stability. Frequency was an important parameter to the electrochemical response, and it also was optimized in Figure 2. In addition, different results emerged using the SWV technique in 0.25 mol L^{-1} H_2SO_4 containing 0.5μmol L^{-1} L-DOPA (dashed line) or D-DOPA (solid line), the SWV peak currents for PEDOT-Boc-Leu of D- and L-DOPA are 34.5 and 44.9μA, and the SWV peak currents for PEDOT-Leu 12.6 and 12.1μA respectively when the scanning potential of SWV was changed from low to high values (forward scan). In contrast, the peak currents for PEDOT-

Boc-Leu are –31.5 and -39.1μA, and the peak currents for PEDOT-Leu are -2.6 and -2.1μA corresponding to D- and L-DOPA respectively with a reverse scan. The peak current values for PEDOT-Boc-Leu of enantiomers are two times larger than the peak current values for the PEDOT-Phe of enantiomers either in the forward or reverse scanning. So the difference in SWV peak currents may be sufficient to enable an accurate determination of the enantiomeric purity and composition of the DOPA analyte [9].

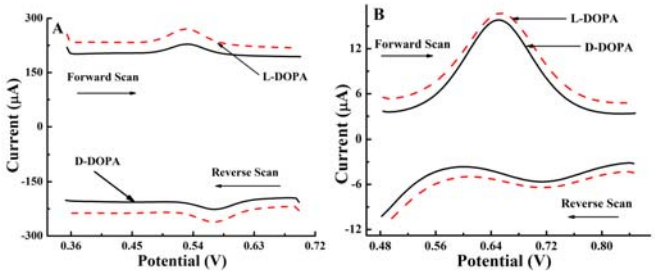

Figure 3. Square wave voltammetrys of PEDOT-Boc-Leu (A) and PEDOT-Leu (B) modified electrodes in 0.25 mol L^{-1} H_2SO_4 containing 0.5μmol L^{-1} L-DOPA (dashed line) or D-DOPA (solid line). Square wave voltammetry: step height, 0.004 V; frequency, 15 Hz.

In 0.25 mol L^{-1} H_2SO_4 containing 0.5μmol L^{-1} L-DOPA (dashed line) or D-DOPA (solid line), the DOPA enantiomers showed different SWV behaviours and thus enabled the sensor to convert the enantioselective recognition event into current changes. Basically, DOPA in the solution should exchange electrons with the GCE electrode after passing through the recognition layer. The peak current values for PEDOT-Boc-Leu were larger than PEDOT-Leu's, and this result may be attributed to the special structure of PEDOT-Boc-Leu, which might accelerate the electron transfer rate between PEDOT-Boc-Leu and DOPA in the solution. The electrochemical activity of DOPA was so good that the enantiomers cannot be distinguished by using common linear sweep voltammetry due to similar peak currents. Consequently, extending the interaction time between the DOPA enantiomers and chiral PEDOT-Boc-Leu and PEDOT-Leu was a key point to success. The waveform of SWV was a staircase scan, each tread of which was superimposed by a symmetrical double pulse, one in the forward direction and the other in the reverse (Figure 3). When SWV was applied in this case, the DOPA enantiomers may pass through the chiral selectivity zone in a manner of oscillation, which would effectively extend the retention time. Each waveform scan made such an interaction time increase a little. After many cycles, the enantioseparation was finally amplified and thus the enantiomers were discriminated. In other words, SWV made limited recognition sites reused, which greatly increased the efficiency of chiral recognition. Meanwhile, PEDOT-Boc-Leu and PEDOT-Leu also acted as bidirectional switches, so the peak currents of D- and L-DOPA were different with different potential scanning directions.

IV. Conclusion

In this study, chiral PEDOT–Boc–Leu and PEDOT–Leu modified electrodes were prepared by a simple electrochemical method and used to distinguish DOPA enantiomers with the help of sulphuric acid. DOPA enantiomers were successful identified by chiral PEDOT–Boc–Leu and PEDOT–Leu modified electrodes, and SWV played a significant role in this sensing strategy. The features of the approach were simplicity, rapidity and sensitivity. The method played a vital role for the chiral recognition of various biomolecules.

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References

[1] P. B. Messersmith, "Multitasking in tissues and materials," Science, vol. 319, pp. 1767-1768, 2008.

[2] L. B. Liu, L. Burdine, and T. Kodadek, "Chemistry of periodate-mediated cross-linking of 3,4-dihydroxylphenylalanine containing molecules to proteins," J. Am. Chem. Soc., vol. 128, pp. 15228–15230, 2006.

[3] W. Poewe, A. Antonini, J. C. Zijlmans, P. R. Burkhard, and F. Vingerhoets, "Levodopa in the treatment of Parkinson's disease: an old drug still going strong," Clin. Interv. Aging, vol. 5, pp. 229–238, 2010.

[4] G. Pezzoli and M. Zini, "Levodopa in Parkinson's disease: from the past to the future," Expert Opin. Pharmacother, vol. 11, pp. 627–635, 2010.

[5] G. Cotzias, C. P. Papavasi, and R. Gellene, "Modification of Parkinsonism—Chronic Treatment with L-Dopa," N. Engl. J. Med., vol. 280, pp. 337-345, 1969.

[6] T. Alexander, C. E. Sortwell, C. D. Sladek, R. H. Roth, and K. S. Collier, "Comparison of Neurotoxicity Following Repeated Administration of L-Dopa, D-Dopa, And Dopamine to Embryonic Mesencephalic Dopamine Neurons in Cultures Derived From Fisher 344 And Sprague-Dawley Donors," Cell Transplant, vol. 6, pp. 309–315, 1997.

[7] A.S.D. Spiers, "Ineffectiveness of dextrodopa in chronic granulocytic leukaemia," Aust. N. Z. J. Med., vol. 4, pp. 475–478, 1974.

[8] J. Moses, A. Siddiqui, and P. B. Silverman, "Sodium benzoate differentially blocks circling induced by D- and L-dopa in the hemi-parkinsonian rat," Neurosci. Lett., vol. 218, pp. 145-148, 1996.

[9] L. S. Chen, F. X. Chang, L. C. Meng, M. X. Li, and Z. W. Zhu, "A novel electrochemical chiral sensor for 3,4-dihydroxyphenylalanine based on the combination of single-walled carbon nanotubes, sulfuric acid and square wave voltammetry," Analyst, vol. 139, pp. 2243-2248, 2014.

[10] R. Török, R. Berkecz, and A. Péter, "Enantioseparation of phenylalanine analogs on a quinine-based anion-exchanger chiral stationary phase: structure and temperature effects," J. Sep. Sci., vol. 29, pp. 2523–2532, 2006.

[11] K. McMurtrey, C. Strawbridge, and J. McCoy, "HPLC resolution of the enantiomers of dihydroxyphenylalanine and selected salsolinol derivatives using sulfated bcyclodextrin," Enantiomer, vol. 5, pp. 377–383, 2000.

[12] J. Wang and Y. Fang, "Determination, purity assessment, and chiral separation of levodopa methyl ester in bulk and formulation pharmaceuticals," Biomed. Chromatogr., vol. 20, pp. 904–910, 2006.

[13] A. Ghassempour, R. Alizadeh, N.M. Najafi, A. Karami, A. Römpp, and B. Spengler et al., "Crystalline degradation products of vancomycin as chiral stationary phase in microcolumn liquid chromatography," J. Sep. Sci., vol. 31, pp. 2339–2345, 2008.

[14] M. Blanco and I. Valverde, "Chiral and non-chiral determination of DOPA by capillary electrophoresis," J. Pharm. Biomed. Anal., vol. 31, pp. 431–438, 2003.

[15] M. Dolezalová and S. Fanali, "Enantiomeric separation of dihydroxyphenylalanine (DOPA), methyldihydroxyphenylalanine (MDOPA), and hydrazinomethyldihydroxyphenylalanine (CDOPA) by using capillary electrophoresis with sulfobutyl ether- b-cyclodextrin as a chiral selector," Electrophoresis, vol. 21, pp. 3264–3269, 2000.

[16] S. La, S. Ahn, J.H. Kim, J. Goto, O.K. Choi, and K.R. Kim, "Enantioseparation of chiral aromatic amino acids by capillary electrophoresis in neutral and charged cyclodextrin selector modes," Electrophoresis, vol. 23, pp. 4123–4131, 2002.

[17] S. Wongwan, M. Hammitzsch-Wiedemann, and G.K.E. Scriba, "Determination of related substances of levodopa including the R-enantiomer by CE," Electrophoresis, vol. 30, pp. 3891–3897, 2009.

[18] P.J. Ream, S.W. Suljak, A.G. Ewing, and K.A. Han, "Micellar electrokinetic capillary chromatography–electrochemical detection for analysis of biogenic amines in Drosophila melanogaster," Anal. Chem., vol. 75, pp. 3972–3978, 2003.

[19] C. Borst and U. Holzgrabe, "Enantioseparation of dopa and related compounds by cyclodextrin-modified microemulsion electrokinetic chromatography," J. Chromatogr. A, vol. 1204, pp. 191–196, 2008.

[20] M.A. Schwarz and P.C. Hauser, "Chiral on-chip separations of neurotransmitters," Anal. Chem., vol. 75, pp. 4691–4695, 2003.

[21] M. Johirul, A. Shiddiky, R.E. Kim, and Y.B. Shim, "Microchip capillary electrophoresis with a cellulose–DNA-modified screen-printed electrode for the analysis of neurotransmitters," Electrophoresis, vol. 26, pp. 3043–3052, 2005.

[22] G. Heywang and F. Jonas, "Poly(alkylenedioxythiophene)s—new, very stable conducting polymers," Adv. Mater., vol. 4, pp. 116-118, 1992.

[23] F. Jonas and G. Heywang, "Technical applications for conductive polymers," Electrochim. Acta, vol. 39, pp. 1345–1347, 1994.

[24] K. Neyts, A. Real, M. Marescaux, S. Mladenovski, and J. Beeckman, "Conductor grid optimization for luminance loss reduction in organic light emitting diodes," J. Appl. Phys., vol. 103, pp. 093113, 2008.

[25] F. Jonas, W. Krafft, and B. Muys, "Poly(3, 4-ethylenedioxythiophene): Conductive coatings, technical applications and properties," Macromol. Symp., vol. 100, pp. 169-173, 1995.

[26] F. Jonas and J. T. Morrison, "3,4-polyethylenedioxythiophene (PEDT): Conductive coatings technical applications and properties," Synth. Met., vol. 85, pp. 1397–1398, 1997.

[27] E. L. Williams, G. E. Jabbour, Q. Wang, S. E. Shaheen, D. S. Ginley, and E. A. Schiff, "Conducting polymer and hydrogenated amorphous silicon hybrid solar cells," Appl. Phys. Lett., vol. 87, pp. 223504, 2005.

[28] J. G. Chen, H. Y. Wie, and K. C. Ho, "Using modified poly(3,4-ethylene dioxythiophene): Poly(styrene sulfonate) film as a counter electrode in dye-sensitized solar cells," Sol. Energy Mater. Sol. Cells, vol. 91, pp. 1472–1477, 2007.

[29] D. M. Welsh, L. J. Kloeppner, L. Madrigal, M. R. Pinto, B. C. Thompson, and K. S. Schanze et al., D. Powell and J. R. Reynolds, "Regiosymmetric dibutyl-substituted poly(3,4-propylenedioxythiophene)s as highly electron-rich electroactive and luminescent polymers," Macromolecules, vol. 35, pp. 6517–6525, 2002.

[30] A. Cirpan, A. A. Argun, C. R. G. Grenier, B. D. Reeves, and J. R. Reynolds, "Electrochromic devices based on soluble and processable dioxythiophene polymers," J. Mater. Chem., vol. 13, pp. 2422–2428, 2003.

[31] P. Schottland, K. Zong, C. L. Gaupp, B. C. Thompson, C. A. Thomas, and I. Giurgiu et al., "Poly(3,4-alkylenedioxypyrrole)s: highly stable electronically conducting and electrochromic polymers," Macromolecules, vol. 33, pp. 7051–7061, 2000.

[32] D.F. Hu, B.Y. Lu, X.M. Duan, J.K. Xu, L. Zhang, and K.X. Zhang et al., "Synthesis of novel chiral L-leucine grafted PEDOT derivatives with excellent electrochromic performances," RSC Adv., vol. 4, pp. 35597–35608, 2014.