Journal of the Korean Electrochemical Society
Vol. 7, No. 2, 2004, 83-88

Amperometric Detection of Some Catechol Derivatives and *o*-aminophenol Derivative with Laccase Immobilized Electrode: Effect of Substrate Structure

De Quan and Woonsup Shin†

Department of Chemistry, Sogang University, Seoul, 121-742, Korea

(Received April 12, 2004 : Accepted May 4, 2004)

Abstract : DeniLite™ laccase immobilized Pt electrode was used for amperometric detection of some catechol derivatives and *o*-aminophenol (OAP) derivative by means of substrate recycling. In case of catechol derivatives, the obtained sensitivities are 85, 79 and 57 nA/μM with linear ranges of 0.6~30, 0.6~30 and 1~25 μM and detection limits (S/N=3) of 0.2, 0.2 and 0.3 μM for 3,4-dihydroxycinnaminic acid (3,4-DHCA), 3,4-dihydroxybenzoic acid (3,4-DHBA) and 3,4-dihydroxyphenylacetic acid (3,4-DHPAA), respectively. In case of OAP derivative, the obtained sensitivity is 237 nA/μM with linear range of 0.2~15 μM and detection limit of 70 nM for 2-amino-4-chlorophenol (2-A-4-CP). The response time ($t_{90\%}$) is about 2 seconds for each substrate and the long-term stability is around 40~50 days for catechol derivatives and 30 days for 2-A-4-CP with retaining 80% of initial activity. The optimal pHs of the sensor for these substrates are in the range of 4.5~5.0, which indicates that stability of the enzymatically oxidized product plays a very important role in substrate recycling. The different sensitivity of the sensor for each substrate can be explained by the electronic effect of the substituent on the enzymatically oxidized form.

Key words : Laccase, Amerometric biosensor, Catechol derivatives, 2-Amino-4-chlorophenol, Substrate recycling

1. Introduction

Determination of catechol derivatives is important in food chemistry, neurochemistry, and environmental chemistry. Several detection methods for catechol derivatives have been reported, for example, IR, HPLC, chromatography, chemiluminescent (CL), electrochemiluminescent (ECL), etc.[1] Besides these instrumental analysis methods, biosensor method was also reported for these detections.[2-4] The method has advantages in easy fabrication, fast analysis, low-cost, etc. Laccase from *T. versicolor* or *C. hirsutus* based biosensors fabricated on carbon electrodes were reported to detect catechol derivatives such as 3,4-dihydroxyphenylacetic acid (3,4-DHPAA, also known as DOPAC),[3-5] 3,4-dihydroxybenzoic acid (3,4-DHBA) and *o*-aminophenol (OAP).[4] Other enzymes such as cellobiose dehydrogenase (CDH, *P. chrysosporium*) and horseradish peroxidase (HRP) were also used for detection of 3,4-DHPAA, 3,4-DHBA and 3,4-dihydroxyhydrocinnamic acid (DHHCA)[6] or 3,4-DHBA.[7] 3,4-DHPAA is known as a structurally related metabolite of dopamine,[3] which is a well-known neurotransmitter. 2-Amino-4-chlorophenol (2-A-4-CP) is a derivative of OAP and belongs to chlorophenols (CP), which constitute a major class of organic pollutants. Amperometric detection of CP with a biosensor system using chloroperoxidase and glucose oxidase was reported,[8,9] and amperometric detections of 2-A-4-CP with laccase (*C. hirsutus*)[7] or HRP[10] immobilized sensor were also reported.

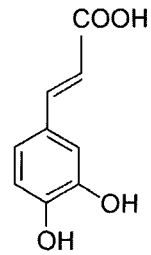
Laccase (*p*-diphenol:dioxygen oxidoreductase, EC 1.10.3.2) is the most promising enzyme to detect phenol derivatives in terms of detection principles and stability. Laccase is a blue multi-copper containing enzyme, which catalyzes the oxidation of a variety of organic substrates such as phenols coupled to the reduction of molecular oxygen to water.[11-14] Laccase based biosensors were reported to detect catecholamines by means of substrate recycling.[13-15] The recycling system used in current study is enzymatic oxidation of substrate followed by electrochemical regeneration as we already reported.[16] The more stable the enzymatically oxidized product the greater amperometric detection signal will be obtained.

We recently reported that laccase purified from DeniLite™ could be successfully covalently immobilized on platinum electrode[17,18] and the electrode can be used as a biosensor for detection of *p*-phenylenediamine (PPD), *p*-aminophenol (PAP)[16] and catechol, catecholamines.[19] In this paper, we describe the application of the sensor for detection of some catechol derivatives and *o*-aminophenol derivative (Fig. 1) and show the relationship between substrate structure and sensitivity of the sensor.

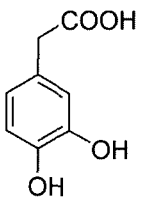
2. Experimental

3,4-Dihydroxycinnaminic acid (97%), 3,4-dihydroxybenzoic acid (97%), 3,4-dihydroxyphenylacetic acid (98%), *o*-aminophenol (99%), 2-amino-4-chlorophenol (97%), *p*-phenylene-

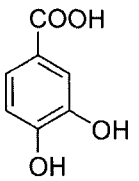
†E-mail: shinws@sogang.ac.kr



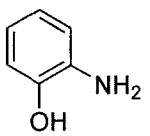
3,4-Dihydroxycinnamic acid
(3,4-DHCA)



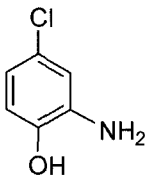
3,4-Dihydroxyphenylacetic acid
(3,4-DHPAA or DOPAC)



3,4-Dihydroxybenzoic acid
(3,4-DHBA)



o-Aminophenol
(OAP)



2-Amino-4-chlorophenol
(2-A-4-CP)

Fig. 1. **Structures of selected catechol derivatives and OAP derivative.**

diamine (98%), 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS, 98%), 3-aminopropyltriethoxysilane (APTES, 99%), glutaraldehyde (25% aqueous solution) and bovine serum albumin (BSA, 98%) were purchased from Sigma & Aldrich Co. and used without further purification. Other chemicals were of analytical grade. Deionized water (18 M cm) from Milli Q water purification system was used for preparing buffer and stock solutions. The stock solutions of the substrates were prepared just before use and kept in the dark enclosed by aluminum foil under argon until the measurements.

McIlvaine buffer (0.05 M citric acid/0.1 M Na_2HPO_4, pH 2.5~8.0) was used for pH dependence studies. For the activity measurement of the purified laccase, MES buffer (8 mM, pH 5.3) was used. For sensor experiments, 0.05 M phosphate buffer solution (PBS, Na_2HPO_4 /KH_2PO_4) and McIlvaine buffer were used.

Platinum disk working (ϕ 4 mm), platinum wire counter (spiral), and Ag/AgCl (3 M KCl) reference electrodes were used for electrochemical measurements. BAS 50 W or cDAQ-1604 (Elbio Co., Korea) potentiostat was used to run CVs and measure current-time responses. The electrochemical cell (5 mL in volume) was equipped in a thermostatic water bath (25°C) and buffer solution was continuously stirred by magnetic bar during amperometric experiments.

Laccase was isolated and purified from DeniLite™, which is a commercial product for decolorization of indigo dye from Novo Nordisk Co., according to the published method.[17-20] The specific activity is 65 units/mg for ABTS oxidation at room temperature.[21]

The covalent immobilization of laccase was done according to the method we previously reported[16] with a little modification. In this study, the oxidation of Pt electrode surface was done by electrochemical oxidation at +1150 mV *vs.* Ag/AgCl for 20 minutes in 1 N H_2SO_4 solution under argon.[22] The amount of immobilized enzyme was fixed by applying 5 μL of 6 mg/mL enzyme solution.[16] The laccase immobilized electrode was stored in 0.05 M PBS, pH 6.0 at 4 when not in use.

BSA was also immobilized with the same method to test the substrate recycling efficiency.[16] The working potentials in amperometric experiments with laccase immobilized electrode were applied at 100 mV negative than reduction peak potentials of the substrates at corresponding pHs. The sensor signals were normalized by that of PPD to compensate the variations resulted from different fabrications of the enzyme electrodes.

3. Results and discussion

3.1. Detection of catechol derivatives

3.1.1. Sensitivities of the sensor effect of substrate structure

Fig. 2 shows the typical steady state current responses of the laccase immobilized Pt electrode to successive 2.5 μM concentration increments at pH 4.5, 5.0, and 5.0 for 3,4-DHCA, 3,4-DHBA and 3,4-DHPAA, respectively. These pHs are optimal for the respective substrate detection and will be discussed further in the following section. The applied working potentials in amperometric experiments are +100, +50 and 50 mV (vs. Ag/AgCl) for 3,4-DHCA, 3,4-DHBA, and 3,4-DHPAA, respectively. Under these potentials the enzymatically oxidized products for the respective substrates will be 99.99% re-reduced, and consequently "substrate recycling", which was employed as the detection principle in this study, will be effectively occurred. The obtained sensitivities are 85, 79, and 57 nA/μM for 3,4-DHCA, 3,4-DHBA and 3,4-DHPAA, respectively (average of seven detections). From the detection principle, the more stable the enzymatically oxidized product

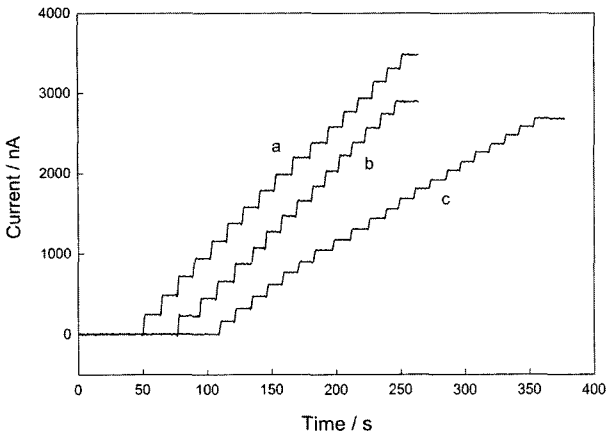


Fig. 2. Steady state current responses of the laccase immobilized Pt electrode to successive 2.5 μM increments for (a) 3,4-DHCA (b) 3,4-DHBA (c) 3,4-DHPAA in McIlvaine buffer. The applied potentials are +100, +50 and 50 mV *vs.* Ag/AgCl for (a), (b) and (c), respectively.

the higher sensitivity will be obtained. If life-time of the oxidized product is short, it will be deactivated before participating in the electrochemical reduction, i.e. half of the whole cycling is blocked to some extent. It is known that the initial product by enzymatic oxidation by laccase is a phenolic radical,[12] which is unstable in aqueous solution depending on pH, ionic strength, type of anions, the availability of nucleophiles, and substrate concentration. The final products of the oxidation are polymerized insoluble materials.[23-27] The stability of the initial radical form will govern the sensitivity of the amperometirc sensor and the stability will be affected by various kinds of electronic effects from the substituents. The more conjugation by the substituents increases the stability of the radical. Also, captodative effect, which is a combined action of an electron-withdrawing and an electron-donating substituent on a radical center, leads to an enhanced stabilization.[28] As shown in Fig. 1, the catechol derivatives have common electron-donating hydroxyl groups and an electron-withdrawing carboxylic acid group and the enzymatically oxidized radical will be stabilized by captodative effect. But the carboxylic group is connected directly to catechol ring for 3,4-DHBA and one-carbon apart for 3,4-DHPAA. It is linked to the ring by conjugation in case of 3,4-DHCA. Therefore, the radical stabilization effect will be the highest for highly conjugated structure and the stabilities of the enzymatically oxidized forms should be in decreasing order of 3,4-DHCA > 3,4-DHBA > 3,4-DHPAA, and consequently the sensitivities of the sensor for these substrates should also be in the same order. The obtained sensitivities of the sensor are in well accordance with structural differences among these substrates.

The sensitivities for catechol derivatives are less than half that for catechol (210 nA/μM)[19] and this difference can be explained by different accessibility of these substrates to the hydrophobic active site of the enzyme since catechol bears a neutral charge and other catechol derivatives having carboxylic group possess partial negative charge in the tested solution. Comparatively, the reported sensitivities of *T. versicolor* laccase adsorbed graphite electrode are 69, 34 and 87 nA/μM and relative sensitivity of *C. hirsutus* laccase immobilized sensor are 100%, 54% and 75% for catechol, 3,4-DHBA and 3,4-DHPAA, respectively.[4] In another case, the relative sensitivity of CDH adsorbed graphite electrode is 100%, 45%, 35% and 32% for catechol, 3,4-DHHCA, 3,4-DHBA, and 3,4-DHPAA, respectively.[6] The relative sensitivity of the sensor reported here is 100%, 40%, 38% and 27% for catechol, 3,4-DHCA, 3,4-DHBA and 3,4-DHPAA, respectively, which is very similar to that of the CDH immobilized sensor. These differences between catechol and its derivatives are mainly attributed to the different substrate specificity of the different sources of laccases and the different enzyme immobilization methods.

3.1.2. Responses of the sensor for selected catechol derivatives

As can be seen in Fig. 2, the sensor signals for catechol derivatives are very stable and the responses are very fast. The response times ($t_{90\%}$) are no more than 2 seconds. The fast response is a clear advantage compared to the relatively long response time (150 seconds) of other reported membrane based recycling system[3] for DOPAC detection where *C. hirsutus* laccase was immobilized on glassy carbon electrode by entrapping in PVA or gelatin membrane. The covalent immobilization could make the redox cycle of the substrate to take place on the surface very closely and minimize a diffusional resistance between the electrode surface and the enzyme layer.[16]

Calibration plots of the sensor for the selected catechol derivatives show the linear response ranges are 0.6~30, 0.6~30 and 1.0~25 μM ($r^2 = 0.999$) and the detection limits (S/N=3) are 0.2, 0.2 and 0.3 μM for 3,4-DHCA, 3,4-DHBA and 3,4-DHPAA, respectively (data not shown). These data are comparable to those of *T. vericolor* laccase adsorbed graphite electrode in which the linear responses are 1~20 and 1~10 μM and the detection limits are 0.66 and 0.13 μM for 3,4-DHBA and 3,4-DHPAA.[4] Detection limits of CDH immobilized sensor were reported to be 3.5, 13 and 18 nM for 3,4-DHHCA, 3,4-DHBA and 3,4-DHPAA, respectively,[6] where flow injection analysis (FIA) method was employed.

The relative standard deviations (R.S.D) of the current response for successive measurements (n=7) in the same detection are 3.1%, 3.4% and 4.0%, and the amplification factors (A.F.) are 12, 11 and 8 for 3,4-DHCA, 3,4-DHBA, and DOPAC, respectively. We reported that A.F. of the sensor for catechol was 17.[19] The A.F. values are consistent with sensitivities of the sensor for these substrates. These data are comparable to those of CDH immobilized sensor, i.e. 14, 12, 8 and 7 for catechol, 3,4-DHHCA, 3,4-DHBA, and DOPAC, respectively.[6] The long-term stability of the sensor for the catechol derivatives is about 40~50 days with retaining 80% of initial activity. It was reported that stability of the CDH immobilized sensor was only 1 week with retaining 50% of initial activity.[6] Obviously, the improved long-term stability of the sensor reported here results from the stable enzyme loading due to the covalent immobilization of the enzyme. The fast response and durable long-term stability of the sensor are principal advantages over those of reported.

3.1.3. pH dependence of the sensor

Fig. 3 shows pH dependences of the sensor for the selected catechol derivatives. The obtained pH profiles are bell-shaped and the optimal pHs are 5.0 for 3,4-DHBA and 3,4-DHPAA, and 4.5 for 3,4-DHCA. From the principle of substrate recycling employed in this study, sensitivity of the sensor is mainly determined by two factors-enzyme activity and reversibility of the substrate. To check the reversibility, CVs of 3,4-DHCA, 3,4-DHBA and 3,4-DHPAA were run on bare Pt electrode at 50 mV/s scan rate from pH 4 to 7. The reversibilities of these substrates did not show remarkable changes upon changing pHs in this range (data not shown), which indicates that relative stabilities of the oxidized products of these substrates are similar at the tested time window. In case of catechol and catecholamines detection with the same sensor,[19] the optimal pH for catechol was 5.5 and those for catecholamines shifted towards acidic range, which could be explained satisfactorily by cyclization of the side chain at relative higher pH. In case of the selected catechol derivatives, the shifts of optimal pHs towards acidic range compared to that for catechol may be explained from the stability of the enzymatically oxidized product and deprotonation of the carboxylic acid functional group on the catechol ring. It is known that, in aqueous solution, acidic condition is advantageous to stable existence of oxidized product of di-phenols.[16] It is reported that pK_a of 3,4-DHCA is 4.69,[29] and that of 3,4-DHPAA is 4.22,[30] and pK_a of 3,4-DHBA is 4.20. On the other hand, active site of laccase was reported to be composed of hydrophobic patch[12,31] having no acidic group. Therefore, in terms of accessibilities of the substrates to active site of the enzyme alone, the optimal pHs will be smaller than the pK_as of the substrates. If optimal pH of 5.5 for the enzyme function[16] is taken into account at the same time, obtained optimal pHs of 4.5~5.0 for these substrates in this study are reasonable.

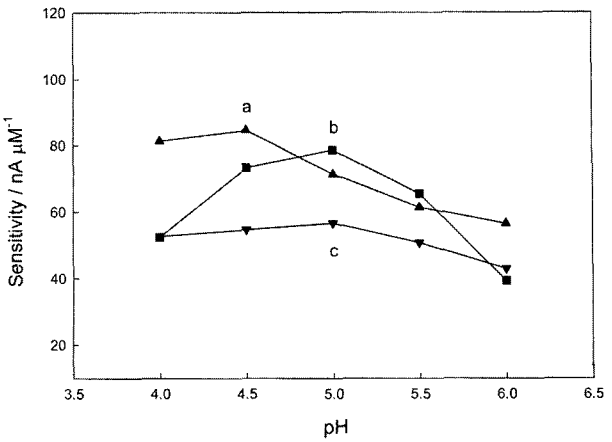


Fig. 3. pH dependences of the sensor in McIlvaine buffer for (a) 3,4-DHCA (b) 3,4-DHBA and (c) 3,4-DHPAA.

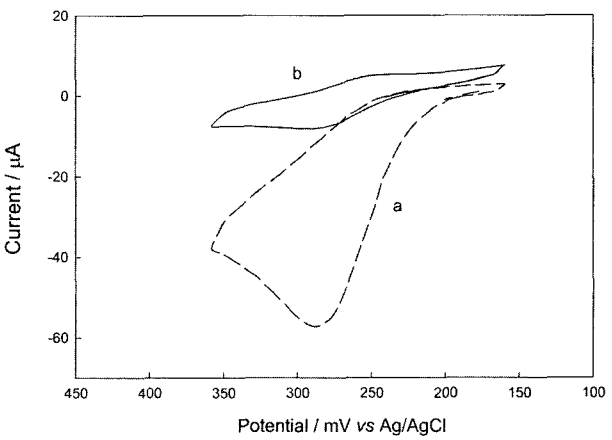


Fig. 4. CVs of different concentrations of 2-amino-4-chlorophenol on bare Pt electrode in McIlvaine buffer, pH 6.0. (a) 10^{-3} M and (b) 10^{-4} M.

3.2. Detection of o-aminophenol derivative

Fig. 4 shows CVs of 2-A-4-CP at different concentrations. While only irreversible oxidation peak is shown at relatively higher concentration of the substrate (10^{-3} M), the re-reduction peak appears at relatively lower concentration of the substrate (10^{-4} M). However, in case of OAP, almost no re-reduction peak could be observed at the same low concentration of 10^{-4} M (data not shown). It is known that OAP is very electro-active, and the oxidized form of the substrate, which is known as *o*-quinoneimine (QI), is unstable in aqueous solution at relative high pH depending on the OAP concentration.[32] Obviously, the electron withdrawing effect of the chloro-group at *para*-position on the benzene ring of 2-A-4-CP keeps the oxidized product relatively stable from polymerization.

To compare further the relative stability of 2-A-4-CP and OAP, the amperometric sensor signals were obtained for both substrates (Fig. 5). The signal for 2-A-4-CP is very stable and the response is fast ($t_{90\%}$~2 seconds) with sensitivity of 237 nA/μM, while the signal for OAP is unstable and noisy with sensitivity of only 21 nA/μM (inset of Fig. 5). For 2-A-4-CP and OAP, since both substrates are in the same neutral charge state, the accessibility capability the accessibility to the active site will not be drastically different each other contrary to catechol with catechol derivatives as we discussed earlier. So the stability of phenolic radical produced by enzymatic oxidation is a main factor for the sensitivity of the sensor. The higher sensitivity of 2-A-4-CP *vs.* OAP is a clear example of captodative effect where the electron-withdrawing -Cl substitute improves the stability of the enzymatically oxidized phenolic radical.

The sensitivity of 2-A-4-CP by our sensor, 237 nA/μM, is well comparable to the reported one, 220~255 nA/μM, where chloroperoxidase and glucose oxidase were immobilized for detecting other CPs,[9] and response time is rather short (2 s *vs.* 100 s). The obtained A. F. value for 2-A-4-CP is 16, which is close to that for catechol. Sensitivity of the sensor for OAP is 21 nA/μM (*vs.* 210 nA/μM for catechol), which is higher than that of reported tyrosinase sensor, i.e. 0.7 nA/μM (*vs.* 34 nA/μM for catechol).[33]

Calibration curve of the sensor for 2-A-4-CP shows the linear range is 0.2~15 μM ($r^2 = 0.997$) and the detection limit

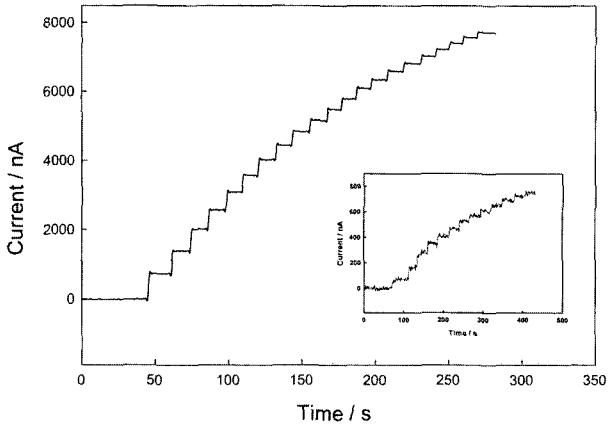


Fig. 5. Steady state current responses of the laccase immobilized Pt electrode to successive 2.5 μM increments for 2-A-4-CP in McIlvaine buffer. The applied potential is +250 mV *vs.* Ag/AgCl. Inset: Response to successive 5.0 μM increments for OAP in the same buffer. The applied potential is +200 mV *vs.* Ag/AgCl.

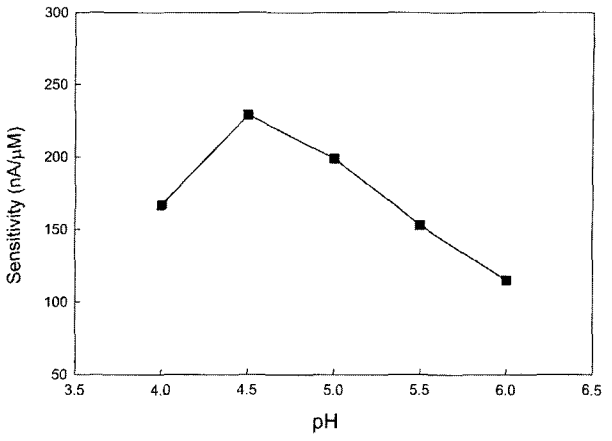


Fig. 6. pH dependence of the sensor in McIlvaine buffer for 2-A-4-CP.

is 70 nM (S/N = 3), which is better than the reported one, i.e. 500 nM with HRP immobilized sensor.[10] The long-term stability is about 30 days for 2-A-4-CP with retaining 80% of initial activity. It was reported that sensor for other CPs detection could be used for only 60 repeated analysis without losing activity.[9]

Fig. 6 shows the pH dependence of the sensor for 2-A-4-CP where optimal pH 4.5 is obtained. We cannot compare this data with others because there is no corresponding study. This optimal pH can also be explained from the stability of the oxidized product and the enzymes functional stability. As mentioned in Section 3.1.3, acidic condition is advantageous to stable existence of the oxidized product of di-phenols. On the other hand, $pK_a(\text{-}NH_2)$ value for OAP was reported to be around 4.65~4.78,[34,35] and that for 2A-4-CP will further decrease compared to this value due to the electron-withdrawing effect of Cl substitute at *para* position of the benzene ring. Therefore, 2A-4-CP will mainly exist in neutural form at pH ~4.5 or greater, which is advantageous for access of the substrate to the active site of laccase. Furthermore, the optimal pH for DeniLite™ laccase function is 5.5~6.0.[16] Therefore, the optimal pH occurred at 4.5 will be a combination of these three factors to give balance of the two opposing effects as that of catecholamines case.[19]

4. Conclusions

The DeniLite™ laccase covalently immobilized sensor shows fast response and durable long-term stability for the catechol derivatives and OAP derivative. Detection limit for 2-amino-4-chlorophenol is in submicro-molar level. Electronic effects of the substitutes can be used to explain the sensitivity differences among catechol derivatives and that between *o*-aminophenol and 2-amino-4-chlorophenol. pH studies show that enzyme activity, stability of oxidized product of the substrates and accessibility of the substrates to the active site of the enzyme are the key factors that decide the sensors sensitivity.

Acknowledgments

The authors acknowledge Prof. Bongjin Moon for helpful discussion and the financial support from the Ministry of Information and Communication of Korea by grants from the contribution of Advanced Backbone IT Technology Development Project (IMT 2000-B3-2).

References

1. Y. Sun, H. Cui, Y. Li and X. Lin, *Talanta*, **53**, 661 (2000).
2. A. L. Ghindilis, V. P. Gavrilova and A. I. Yaropolov, *Biosens. Bioelectron.*, **7**, 127 (1992)
3. F. Lisdat, U. Wollenberger, A. Markower, H. Hortnagl, D. Pfeiffer and F. W. Scheller, *Biosens. Bioelectron.*, **12**(12), 1199 (1997).
4. B Haghighi, L. Gorton, T. Ruzgas and L. J. Jonsson, *Anal. Chim. Acta*, **487**, 3 (2003).
5. F. Lisdat, W. O. Ho, U. Wollenberger, F. W. Scheller, T. Richter and U. Billitewski, *Electroanalysis*, **10**(12), 803 (1998).
6. A. Lindgren, L. Stoica, T. Ruzgas, A. Ciucu and L. Gorton, *Analyst*, **124**, 527 (1999).
7. G. Marko-Varga, J. Emneus, L. Gorton and T, Ruzgas, *Trends Anal. Chem.*, **14**(7), (1995) 319.
8. S. Zhao and J. H. T. Luong, *Anal. Chim. Acta*, **327**, 235 (1996).
9. C. Saby and J. H. T. Luong, *Electroanalysis*, **10**(1), 7 (1998).
10. T. Ruzgas, E. Csbregi, J. Emneus, L. Gorton and G. Marko-Varga, *Anal. Chim. Acta*, **330**, 123 (1996).
11. B. Reinhammar, in: Copper proteins & copper enzymes (Ed: L. Rene), Chap. 1, Vol. III, CRC Press (1984).
12. E. I. Solomon, U. M. Sundaram and T. E. Machonkin, *Chem. Rev.*, **96**, 2563 (1996).
13. C. F. Thurston, *Microbiology*, **140**, 19 (1994).
14. A. I. Yaropolov, O. V. Skorobogatko, S. S. Vartanov and S. D. Varfolomeyev, *Appl. Biochem. Biotech.*, **49**, 257 (1994).
15. T. Wasa, K. Akimoto, T. Yao and S. Murao, *Nippon Kagaku Kaishi*, **9**, 1398 (1984).
16. D. Quan, Y. Kim and W. Shin, *J. Electroanal. Chem.*, **561**, 181 (2004).
17. D. Quan, Y. Kim, K. Yoon and W. Shin, *Bull. Korean Chem. Soc.*, **23**, 385 (2002).
18. D. Quan and W. Shin, *Materi. Sci. Eng. C*, **24**, 113 (2004).
19. D. Quan and W. Shin, *Electroanalysis*, in press.
20. Y. Kim, N. Cho, T. Eom and W. Shin, *Bull. Korean Chem. Soc.*, **23**,

985 (2002).
21. F. Xu, W. Shin, S. H. Brown, J. Wahleithner, U. M. Sundaram and E. I. Solomon, *Biochim. Biophys. Acta*, **1292**, 303 (1996).
22. J. R. Lenhard and R. W. Murray, *J. Electroanal. Chem.*, **78**, 195 (1977).
23. W. G. C. Forsyth, V. C. Quesnel, J. B. Roberts, *Biochim. Biophys. Acta*, **37**(2), 322 (1960).
24. C. R. Dawson, and W. B. Tarpley, *Ann. N. Y. Acad. Sci.*, **100**(Pt. 2), 937 (1963).
25. R. Z. Kazandjian and A. M. Klibanov, *J. Am. Chem. Soc.*, **107**, 5448 (1985).
26. J. Davis, D. H. Vaughan and M. F. Cardosi, *Electrochim. Acta*, **43**(3-4), 291 (1998).
27. S. Kobayashi and H. Higashimura, *Prog. Polym. Sci.*, **28**, 1015 (2003).
28. H. G. Viehe, Z. Janousek, R. Merenyi and L. Stella, *Acc. Chem. Res.*, **18**, 148 (1985).
29. W. P. Price, Jr., R. Edens, D. L. Hendrix and S. N. Deming, *Anal. Biochem.*, **93**, 233 (1979).
30. T. Ishimitsu, S. Hirose and H. Sakurai, *Talanta*, **24**, 555 (1977).
31. V. Ducros, A. M. Brzozowski, K. S. Wilson, S. H. Brown, P. Ostergaard, P. Schneider, D. S. Yaver, A. H. Pedersen and G. J. Davis, *Nature struct Biol.*, **5**(4), 310 (1998).
32. V. D. Parker, *Anodic oxidation of Amines*, in: M. M. Baizer (Ed), *Organic Electrochemistry-An Introduction and a Guide*, Chap. 14, Marcel Dekker, Inc., New York (1973).
33. S. Campuzano, B. Serra, M. Pedrero, F. J. Manuel de Villena and J. M. Pingarron, *Anal. Chim. Acta*, **494**, 187 (2003).
34. J. M. Vandenbelt, C. Henrich and S. G. Vandenberg, *Anal. Chem.*, **25**(4), 726 (1954).
35. D. C. Harris, *Quantitative chemical analysis*, 5th Ed., AP 15, W. H. Freeman and Company, New York (1999).