Probing of enzyme reactions by the biocatalyst-induced association or dissociation of redox labels linked to monolayer-functionalized electrodes†

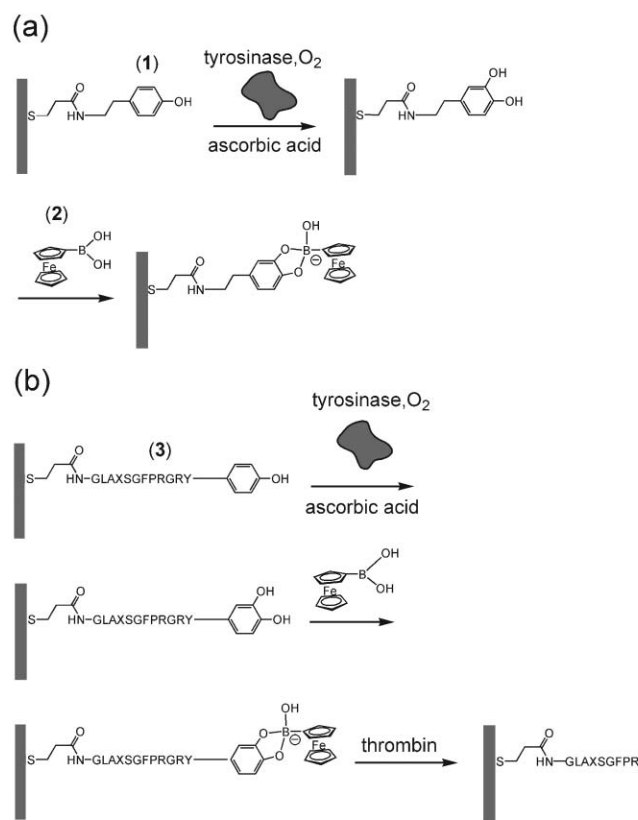
Di Li, Ron Gill, Ronit Freeman and Itamar Willner*

Received (in Cambridge, UK) 28th September 2006, Accepted 2nd November 2006
First published as an Advance Article on the web 20th November 2006
DOI: 10.1039/b614141b

The activities of the enzymes tyrosinase and thrombin are probed by the association of the ferrocene boronic acid label to the enzyme-generated catechol ligand, and by the cleavage of the ligand–redox complex tethered to a peptide, respectively.

Monolayer-modified electrodes are attracting considerable interest as functional surfaces.[1,2] Different uses of monolayer-functionalized electrodes have been reported, and examples include the control of surface hydrophilicity/hydrophobicity,[3] different sensor applications using receptor-modified electrodes,[4,5] the design of photoisomerizable monolayer-functionalized electrodes for information storage and processing,[6] and the organization of supramolecular monolayer assemblies acting as machines.[7] The probing of enzyme activities using nanoscale labels has attracted recent research efforts.[8] The enzyme-induced growth of metallic nanoparticles was used to monitor the biocatalytic functions of enzymes such as glucose oxidase,[9] acetylcholine esterase[10] and tyrosinase.[11] Similarly, the optical assay of hydrolytic enzymes was reported in the presence of CdSe quantum dots functionalized with quencher-tethered peptides.[12] Recently, ferrocene-tethered peptides were immobilized on Au electrodes and used for the electrochemical analysis of matrix metalloproteinase. The electrochemical analysis of the enzyme was then accomplished by the cleavage of the redox-label.[13] In the present study, we report on a method to analyze tyrosinase and thrombin activities by the biocatalytic generation of an active ligand that binds the redox-label and transduces electrochemically the activities of the enzymes.

Tyrosinase, TR, catalyzes the oxidation of tyrosine (or other phenols) to L-DOPA (or catechol derivatives). Elevated amounts of the enzyme appear in melanoma cancer cells,[14] and it is considered to be a biomarker for these cells. The method used to follow the activity of TR is depicted in Scheme 1, path (a). Tyramine, (**1**), was assembled as a monolayer on a Au-electrode, with a surface coverage of 2.75×10^{-10} mol cm^{-2}. The TR-mediated oxidation of tyramine by O_2 yielded the catechol derivative that acted as ligand for the association of ferrocene boronic acid, (**2**), that acted as redox label for the biocatalytic transformation that occurred on the surface. Ferrocene boronic acid exhibits a reversible redox wave at 0.13 V *vs.* SCE. Fig. 1(A) shows the differential pulse voltammograms (DPVs) of the functionalized electrode reacted with TR–O_2, 0.4 units, for variable time-intervals, followed by the interaction with (**2**). As the time-interval for the biocatalytic process is prolonged, the DPV is intensified, implying an increased content of the biocatalytically-synthesized ligand. Fig. 1(B) shows the cyclic voltammograms of the ferrocene-modified electrode at different scan rates. Coulometric analysis of the cyclic voltammogram of the ferrocene units associated with the electrode after 20 minutes of reaction with TR–O_2, 2 units, indicated a surface coverage of the ferrocene sites that corresponded to 3.4×10^{-12} mol cm^{-2}. The linear relation between the peak current and the scan rate, Fig. 1(B), inset, confirms the surface-confined configuration of the redox label. Fig. 2(A) depicts the DPVs corresponding to the ferrocene units associated with the electrodes reacted with different concentrations of TR for a fixed time-interval of 20 minutes. As the concentration



Scheme 1 (a) The analysis of tyrosinase through a redox label that binds to the enzyme-generated ligand. (b) The analysis of tyrosinase and thrombin through the electrochemical response of a redox label associated with or dissociated from a peptide-modified monolayer electrode.

Institute of Chemistry, The Hebrew University of Jerusalem, 91904 Jerusalem, Israel. E-mail: willnea@vms.huji.ac.il; Fax: +972 2 6527715; Tel: +972 2 6585272

† Electronic supplementary information (ESI) available: Experimental section. See DOI: 10.1039/b614141b

This journal is © The Royal Society of Chemistry 2006

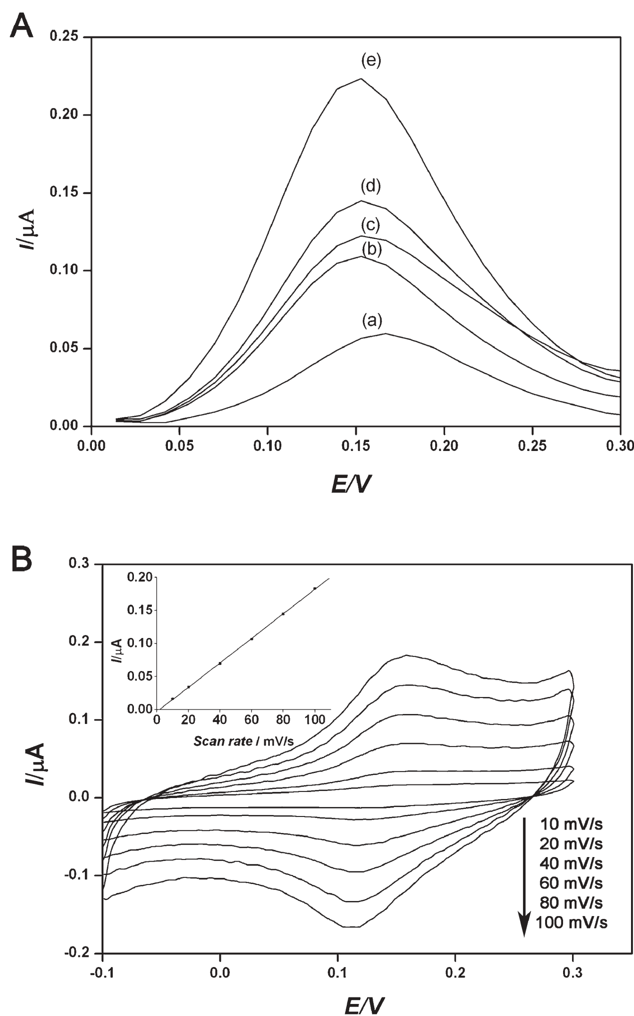


Fig. 1 (A) Differential pulse voltammograms corresponding to the time-dependent increase of the redox label associated with the catechol-functionalized monolayer generated by tyrosinase, 0.4 U at: (a) 0 minutes, (b) 2 minutes, (c) 5 minutes, (d) 10 minutes, (e) 20 minutes. (B) Cyclic voltammograms of the ferrocene label linked to the catechol-ligand-functionalized electrode generated by tyrosinase, 2 units, 20 minutes, at different scan rates. Inset: The plot of the anodic peak current as a function of scan rate.

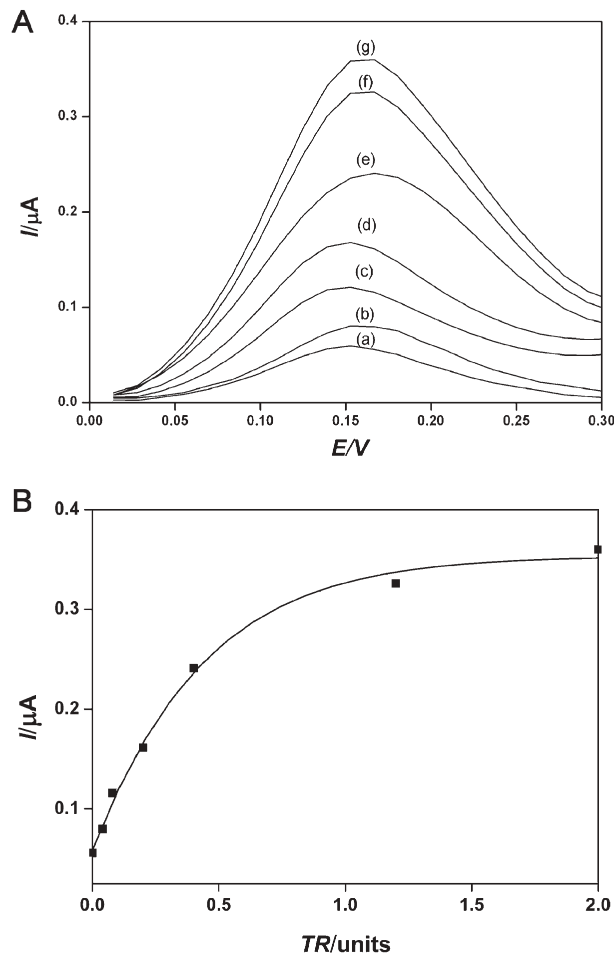


Fig. 2 (A) Differential pulse voltammograms corresponding to the electrical responses of ferrocene redox label associated to the catechol-modified electrode generated by different concentrations of tyrosinase for a fixed time-interval of 20 minutes: (a) 0 units, (b) 0.04, (c) 0.08, (d) 0.2, (e) 0.4, (f) 1.2, (g) 2 units. (B) Calibration curve corresponding to the electrochemical analysis of tyrosinase.

of TR increases the content of the TR-synthesized catechol ligand is higher, and the response of the ferrocene sites is intensified. From the derived calibration curve, Fig. 2(B), we conclude that the sensitivity limit for the detection of TR is 0.04 units. Although detection schemes for TR are few, the present method is *ca.* 200-fold more sensitive than the optical detection of the biocatalytic formation of Au nanoparticles.[11] Furthermore, the calibration curve reveals a minute response of the electrode at [TR] = 0. This is attributed to a residual non-specific adsorption of the ferrocene label to the electrode surface. This adsorption might be blocked by applying surface additives. Also, the coupling of the surface-confined redox mediator with redox enzymes, *e.g.* glucose oxidase,[15] might activate a bioelectrocatalytic cascade that amplifies the analytical procedure, and thus extend the linear detection region to higher sensitivities.

The successful detection of the activity of TR by the electroactive ferrocene label enabled us to sequentially follow the activities of two enzymes: TR and thrombin, Scheme 1, path (b). The tyramine-functionalized peptide (**3**) was immobilized on the Au electrode (surface coverage 3×10^{-11} mol cm^{-2}). Reaction of the chemically-modified electrode with TR–O_2 generated the respective L-DOPA-functionalized peptide that acted as ligand for the subsequent association of (**2**). Fig. 3, curves (a) and (b) show the DPVs of the (**3**)-modified electrode before and after the reaction with TR–O_2 and subsequent labeling with (**2**), respectively. A low intensity DPV is observed before the treatment with TR–O_2, and this is attributed to the non-specific binding of (**2**) to the peptide-functionalized electrode.

After the treatment of the (**3**)-modified electrode with TR–O_2 and labeling with (**2**), the DPV is intensified, implying that the L-DOPA residues were generated on the electrode. The peptide includes the specific sequence for the proteolytic cleavage by thrombin. Indeed, treatment of the TR-synthesized L-DOPA peptide, labeled with (**2**), with thrombin, resulted in the DPV shown in Fig. 3, curve (c). The redox response of (**2**) decreases to the non-specific adsorption value, indicating that (**2**) was cleaved off. Control experiments revealed that the cleavage of the peptide was specific, and treatment of the ferrocene-labeled L-DOPA

This journal is © The Royal Society of Chemistry 2006

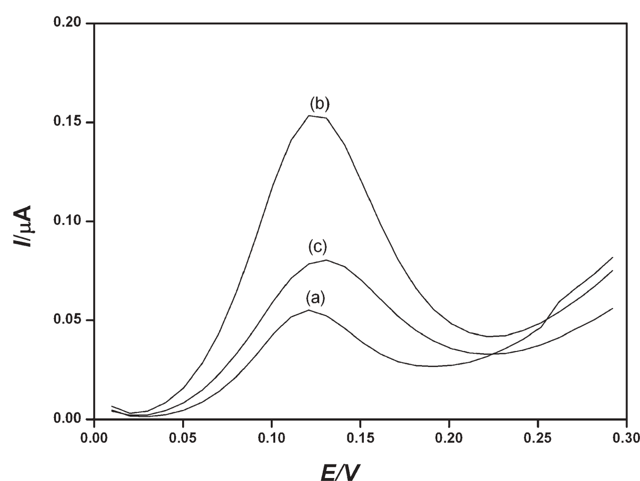


Fig. 3 Differential pulse voltammograms corresponding to: (a) the peptide (**3**)-functionalized electrode that was treated with (**2**) prior to the reaction with TR–O_2, (b) the peptide (**3**)-functionalized electrode that was treated with (**2**) after the reaction with TR (2 units)–O_2 for 20 minutes, (c) after reacting the electrode generated in (b) with thrombin 1×10^{-7} M, for 15 minutes.

peptide with α-chymotrypsin did not affect the electrical response of the ferrocene units, and did not cleave the peptide.

To summarize, the present study has demonstrated the electrochemical transduction of the activities of enzymes by the biocatalytic generation of a catechol ligand on the electrode that is labeled with the electroactive ferrocene unit. The formation and depletion of the ferrocene signals provide the readout signal for the biocatalyzed reactions. One may envisage the biocatalytic generation of other ligands and their imaging with other redox labels as a means to follow other enzymes' activities.

This research is supported by the Israel Ministry of Science as an Infrastructure Project on Nanobiotechnology.

Notes and references

1 J. J. Gooding, F. Mearns, W. R. Yang and J. Q. Liu, *Electroanalysis*, 2003, **15**, 81–96; C. H. Fan, K. W. Plaxco and A. J. Heeger, *Trends Biotechnol.*, 2005, **23**, 186–192.
2 N. K. Chaki and K. Vijayamohanan, *Biosens. Bioelectron.*, 2002, **17**, 1–12; H. O. Finklea, in *Electroanalytical Chemistry*, ed. A. J. Bard and I. Rubinstein, Marcel Dekker, New York, 1996, Vol. 19, pp. 109–335; J. M. Zen, A. S. Kumar and D. M. Tsai, *Electroanalysis*, 2003, **15**, 1073–1087.
3 J. Lahann, S. Mitragotri, T. N. Tran, H. Kaido, J. Sundaram, I. S. Choi, S. Hoffer, G. A. Somorjai and R. Langer, *Science*, 2003, **299**, 371–374; X. M. Wang, A. B. Kharitonov, E. Katz and I. Willner, *Chem. Commun.*, 2003, 1542–1543; M. Riskin, B. Basnar, V. I. Chegel, E. Katz, I. Willner, F. Shi and X. Zhang, *J. Am. Chem. Soc.*, 2006, **128**, 1253–1260.
4 I. Rubinstein, S. Steinberg, Y. Tor, A. Shanzer and J. Sagiv, *Nature*, 1988, **332**, 426–429; S. Flink, F. C. J. M. van Veggel and D. N. Reinhoudt, *Adv. Mater.*, 2000, **12**, 1315–1328.
5 M. Crego-Camala and D. N. Reinhoudt, *Adv. Mater.*, 2001, **13**, 1171–1174; A. B. Descalzo, R. Martinez-Manez, R. Sancenon, K. Hoffmann and K. Rurack, *Angew. Chem., Int. Ed.*, 2006, **45**, 5924–5948.
6 I. Willner and B. Willner, *Adv. Mater.*, 1995, **7**, 587–589; Z. F. Liu, K. Hashimoto and A. Fujishima, *Nature*, 1990, **347**, 658–660; E. Katz and I. Willner, *Chem. Commun.*, 2005, 5641–5643.
7 E. Katz, O. Lioubashevsky and I. Willner, *J. Am. Chem. Soc.*, 2004, **126**, 15520–15532.
8 I. Willner, B. Baron and B. Willner, *Adv. Mater.*, 2006, **18**, 1109–1120.
9 M. Zayats, R. Baron, I. Popov and I. Willner, *Nano Lett.*, 2005, **5**, 21–25.
10 V. Pavlov, Y. Xiao and I. Willner, *Nano Lett.*, 2005, **5**, 649–653.
11 R. Baron, M. Zayats and I. Willner, *Anal. Chem.*, 2005, **77**, 1566–1571.
12 Z. L. Medintz, A. R. Clapp, F. M. Brunel, T. Tiefenbrunn, H. T. Uyeda, E. L. Chang, J. R. Deschamps, P. E. Dawson and H. Mattoussi, *Nat. Mater.*, 2006, **5**, 581–589.
13 G. Liu, J. Wang, D. Wunschel and Y. Lin, *J. Am. Chem. Soc.*, 2006, **128**, 12382–12383.
14 C. Angeletti, V. Khomitch, R. Halaban and D. L. Rimm, *Diagn. Cytopathol.*, 2004, **31**, 33–37.
15 I. Willner and E. Katz, *Angew. Chem., Int. Ed.*, 2000, **39**, 1180–1218.