Chinese Chemical Letters Vol. 15, No. 2, pp 214 – 215, 2004
http://www.imm.ac.cn/journal/ccl.html

Imaging of Activity of Horseradish Peroxidase at β-Cyclodextrin Polymer by Scanning Electrochemical Microscopy

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Abstract: The activity of horseradish peroxidase at β-cyclodextrin polymer was imaged by scanning electrochemical microscopy using 3, 3', 5, 5'-tetramethylbenzide and H_2O_2 as the substrates.

Keywords: Scanning electrochemical microscopy, horseradish peroxidase.

Scanning electrochemical microscopy (SECM) has become a very valuable tool to image biochemical activity of optimizing functionalized surfaces[1]. Recently, Bard's group[2] studied horseradish peroxidase (HRP) immobilized with copolymer on insulating substrates. In their experiments, hydroquinone, which was produced at the tip by reducing 1,4-benzoquinone (BQ) at –0.4 V *vs*. saturated Ag/AgCl, and H_2O_2 were used as the substrates of HRP. Under these conditions, the background current was as high as 10^{-9} A, because of the reduction of oxygen in the solution. If 3, 3', 5, 5' -tetramethylbenzide (TMB_{red}) and H_2O_2 were chosen as the substrates, HRP can catalyze the enzymatic reaction as follows:

$$TMB_{red} + H_2O_2 \xrightarrow{HRP} TMB_{ox} + 2H_2O \qquad (1)$$

The oxidized product TMB_{ox} of the enzymatic catalysis reaction can be reduced at the Pt tip at a positive potential of 0.2 V. *vs*. 1 mol/L Ag/AgCl and convert to TMB_{red}.

$$TMB_{ox} + 2H^+ + 2e^- \longrightarrow TMB_{red} \qquad (2)$$

Using this method, the background current of SECM was reduced to 10^{-11} A. This reaction system could be used to image the activity of horseradish peroxidase at β-cyclodextrin polymer by SECM. In our experiments, the SECM images were measured with a model CHI 900 SECM (CH Instruments, Austin, TX, USA). The method of HRP immobilization was: an aqueous mixture of β-CDP, glutaric dialdehye, HCl, bovine serum albumin and HRP solution were spotted on a plastic plate. Due to

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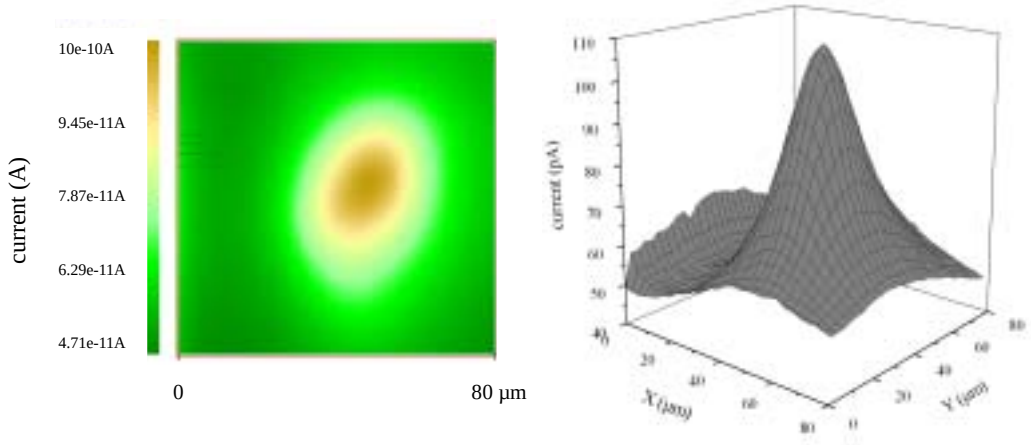
the poly-condensation of those substances, HRP was immobilized on the plate. **Figure 1A** shows the gray-scaled image of the activity of the HRP spot detected in 0.02 mol/L phosphate solution containing 2×10^{-4} mol/L TMB_{red} and 4×10^{-4} mol/L H_2O_2 (pH 7.2). The current in the center of the spot reached to its maximum where the color was gray, and the current became smaller at the farther distance from the center where the color was lighter and lighter. When the tip left the HRP spot, the current was dropped fast where the color was black. In order to display it more clearly, a topographic image of the same spot is shown in **Figure 1B**.

Figure 1 The gray-scaled (A) and topographic (B) image of the activity of a HRP spot immobilized on a plastic plate by SECM



Acknowledgments

This project was supported by The National Natural Science Foundation of China (No. 20235010).

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Received 11 February, 2003