PURIFICATION AND APPLICATION OF FUNGAL HYDROPHOBIN RODA FOR IMPROVEMENT OF GLUCOSE BIOSENSOR

Andrijana Danytė[1], Eimantas Ramonas[1], Jaunius Urbonavičius[1]

[1]Department of Chemistry and Bioengineering, Vilnius Gediminas Technical University, Sauletekio av. 11, LT-10223, Vilnius, Lithuania
andrijana.danyte@vilniustech.lt

Hydrophobins are proteins composed of 70–130 amino acids and containing 8 cysteines, linked by 4 disulfide bonds, which are characteristic of the entire hydrophobin family [1]. The main advantage of hydrophobins is their ability to form amphiphilic layers on various surfaces and thus to change their properties from hydrophilic to hydrophobic and vice versa due to the bifunctional molecular structure of these proteins [2]. Due to their properties, hydrophobins are used in the pharmaceutical, cosmetic and food industries as they are able to stabilize emulsions in product formulation [3]. Hydrophobins are also used in biosensor surface modifications [4] and in tissue engineering for the development of tissue scaffold with higher hydrophilicity [5].

In this work, the hydrophobin RodA of *Aspergillus fumigatus* was investigated as matrix for glucose biosensor. Gene responsible for the synthesis of the RodA was identified, expressed in *Escherichia coli*, and corresponding purified recombinant protein was used as a matrix of gold electrode of the engineered glucose biosensor. The engineered biosensor with a RodA matrix (Au/RodA/GOx) was compared with biosensor without a RodA matrix (Au/GOx), both biosensors had immobilized glucose oxidase (GOx) enzyme. Cyclic voltammetry analysis confirmed the successful immobilization of GOx enzyme for both biosensors and chronoamperometry was used to calculate the K_M values and the maximum generated currents (I_{max}). For Au/GOx, the K_M value was 6.99 mM and the I_{max} was 34.8 $\mu A \cdot cm^{-2}$, K_M value for the Au/RodA/GOx biosensor was 2.37 mM and the I_{max} was 0.432 $\mu A \cdot cm^{-2}$.

The obtained Au/RodA/GOx K_M value showed that GOx immobilized in Au/RodA/GOx biosensor had a higher affinity for the substrate, indicating that hydrophobins are a suitable choice for gold electrode surface modification. The experiments to further improve glucose biosensor are under way.

[1] Hakanpaa, J., Szilvay G.R., & Kaljunen, H. (2006). Two crystal structures of *Trichoderma reesei* hydrophobin HFBI – The structure of a protein amphiphile with and without detergent interaction. Protein Science, 15(9).

[2] Opwis, K., & Gutmann, J.S. (2011). Surface modification of textile materials with hydrophobins. Textile Research Journal, 81(15), 1594-1602.

[3] Hektor, H. J., & Scholtmeijer, K. (2005). Hydrophobins: proteins with potential. Current Opinion in Biotechnology, 16(4).

[4] Zhao, Z., Wang, H., Qin, X., Wang, X., Qiao, M., Anzai, J., & Chen, Q. (2009). Self-assembled film of hydrophoins on gold surfaces and its application to electrochemical biosensing. Colloids and Surfaces B: Biointerfaces, 71(1), 102-106.

[5] Boeuf, S., Throm, T., Gutt, B., Strunk, T., Hoffmann, M., Seebach, E., Mühlberg, L., Brocher, J., Gotterbarm, T., Wenzel, W., Fischer, R., & Richter, W. (2012). Engineering hydrophobin DewA to generate surfaces that enhance adhesion of human cells but not bacterial cells. Acta Biomaterialia, 8(3), 1037-1047.