IMMOBILIZATION OF THE MEMBRANE PROTEIN DsbB ON GOLD AND LIPID BILAYER STABILIZATION

Cigdem Yıldırım¹, Zsófia Keresztes², Judith Mihály², Zoltan Varga², Attila Bota², Ulla Wollenberger³

1 Institute of Biochemistry and Biology, University of Potsdam, Karl-Liebknecht-Str. 24-25, 14476 Golm, Germany
E-mail: Cigdem.Yildirim@uni-potsdam.de

2 Chemical Research Centre, Hungarian Academy of Sciences, Pusztaszeri street, 59-67, 1025 Budapest, Hungary
E-mail: zsofı@chemres.hu

DsbB is one of the main components of the disulphide generation mechanism in E. coli. It has two substrates, one of them is dithiol oxidase (DsbA) that connects DsbB to cellular redox system and the other one is ubiquinone 8 that connects DsbB to the respiratory system. Its function is related with cellular redox status, this makes DsbB a good candidate for a biosensor development about determination of cellular redox status.

For DsbB immobilization gold surfaces were modified with a Ni-NTA carrying monolayer to which his-tagged DsbB was coupled. These steps were investigated with different microscopic and optical methods. Ni-NTA modification could be verified with IR-microscopy. Symmetric and asymmetric stretching peaks of the carboxylate groups of NTA appeared when they were deprotonated during complexation with nickel at 1492 cm⁻¹ and 1572 cm⁻¹, respectively. When DsbB was immobilized on the gold surface the protein amide bonds could be seen by IR-microscopy in the 1658-1571 cm⁻¹ region. Also AFM images show different structures in 2 micrometres scale. After lipid-detergent exchange the surface morphology became more regular and surface roughness was around 10 nm. This corresponds to theoretical value of layer thickness. Activity of DsbB is shown by chronoamperometry.

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References