

Optical resonant mirror biosensor nanochips for investigation of cytochrome P450cam and P4502B4 monooxygenase systems

Yu.D. Ivanov^{*}, N.I. Konstantinova, O.V. Gnedenko, P.A. Lipov, A.O. Viglinskaya, A.I. Archakov

Institute of Biomedical Chemistry RAMS, Laboratory of Biosensor Analysis, 10 Pogodinskaya Ulica, Moscow 119121, Russia.

The optical resonant mirror biosensor nanochips with ≈ 100 nm sensing layer were fabricated for investigation of cytochromes P450cam and P4502B4 (2B4) – monooxygenase biological systems. For this purpose the proteins from P450 systems were immobilized on the optical resonant mirror nanochip surface. These biochips were used for investigation of the interactions in sensing layer between putidaredoxin (Pd), putidaredoxin reductase (PdR) and cytochrome P450cam within P450cam-containing monooxygenase system under hydroxylation conditions in real time. The formation of binary complexes Pd/PdR and Pd/P450cam did occur, with the complex association rate constants for Pd/PdR (k_{on}) = $1.0 \pm .4 \times 10^3 \text{ M}^{-1}\text{s}^{-1}$ and dissociation rate constants (k_{off}) = $0.003 \pm 0.002 \text{ s}^{-1}$, respectively, and for Pd/P450cam with k_{on} = $1.2 \pm .5 \times 10^4 \text{ M}^{-1}\text{s}^{-1}$ and k_{off} = $0.7 \pm 0.3 \text{ s}^{-1}$, respectively. We were carrying out an identification of protein partners from cytochrome P-450 2B4 monooxygenase system with the aid of an optical biochip attended by mass-spectrometer. For this purpose, the protein partners of the cytochrome P450 2B4 system were selectively fishing out on the surface of the optical flavoprotein (Fp)-nanochip from solution. After that the partners were taking away from the biochip and separated by SDS-polyacrylamine gel electrophoresis (SDS-PAGE) for subsequent matrix-assisted laser desorption/ionization time-of-flight (MALDI TOF) mass-spectrometry analysis. This proteomic approaches allows to identify the cytochromes P-450 2B4 fished out on Fp- biochip.

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^{*} Corresponding author. Tel. (7)(095)2461641. FAX (7)(095)2450857.
Email address: yuiv@ibmh.msk.su (Yu.D. Ivanov).