Immobilization of CYP3A4 Enzyme onto SBA-15-like Mesoporous Silica and Porous Silicon Matrices.

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The human cytochrome P450s have essential roles in catalysing reactions involved in the metabolism of endogeneous compounds and drugs [1]. In general, cytochrome P450s act as monooxygenases, catalysing the addition of oxygen to the substrate molecule. Concerning drugs metabolism, an emerging research field focuses on the interactions between foods and drugs. In particular, some foods have been demonstrated to inhibit drugs metabolism operated by cytochrome P450s, resulting in an over-dosage of the drug. Therefore, the investigation about the influence of different food additive or components on P450s enzymatic activity represents a crucial aspect. To this purpose, the immobilization of P450 enzymes (CYP) on solid supports in view of devices for monitoring the enzyme activity either through electrochemical or optical methods is very attractive. In this contribution, the isoenzyme CYP3A4 of cytochrome P450 has been immobilized on SBA-15 mesoporous silica, an optically transparent support, and on porous silicon (PS), a conductive support, with the purpose to characterize the supported enzyme by optical and electrochemical techniques.
Large pores SBA-15 has been prepared by adding 1,3,5 trimethylbenzene (TMB), as swelling agent, to the synthesis mixture [2]. XRD pattern of the calcined silica support shows the reflection peaks typical of hexagonal (p6mm) structure; the cell parameter *a*, calculated from the (100) peak, is 12.6 nm. N_2 adsorption-desorption isotherms are IV type, and the pore size distribution calculated through the DFT method is about 14 nm, *i. e.* able to accommodate the enzyme molecule (3 nm* 5 nm * 7nm). For the enzyme incorporation, the calcined sample has been suspended in a buffer solution (pH = 7.4) containing the enzyme (1μM), the resulting suspension has been shaken for 3 hours at 4°C; then supernatant and powder have been separated by centrifugation. The UV-vis spectrum of the supernatant reveals that all the enzyme has been incorporated onto the silica support. N_2 sorption characterization of the solid suggests the location of the enzyme inside and/or at the openings of mesopores, and a high degree of pore blocking. Diffuse Reflectance UV-vis spectrum of the powder after impregnation (CYP3A4/SBA-15) shows the typical Soret peak of the enzyme. Activity and accessibility of the incorporated enzyme has been tested using erythromycin as substrate and by following the spectral changes in the visible region. Cyclic voltammetry experiments carried out on CYP3A4/SBA-15 modified glassy carbon electrode have shown the occurrence of electron transfer between the enzyme and the electrode.
Porous silicon substrates have been prepared with different thickness, porosity and oxidation degree. For the enzyme incorporation the supports has been soaked in the enzyme solution with different concentration (0.1-10 μM). Electrochemical characterization of immobilized enzyme on PS is in progress.

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