Designing Enzyme Cascades for Cofactor Regeneration

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Due to their high selectivity enzymes are efficient biocatalysts but their application in organic syntheses is expensive since harsh reaction conditions can lead to enzyme denaturation. Therefore researchers try to stabilize enzymes by immobilization on suitable host materials with high retention of activity. Within the last decade mesoporous silicas have established as support material for enzyme immobilization. The pore structure of mesoporous cellular siliceous foams (MCF) is well suited to immobilize larger enzymes, since it consists of a disordered 3-D pore structure with cage-like cells (20-50 nm) interconnected by smaller windows (9-26 nm).[1] Surface modification of the MCF initiates hydrophobic, polar or covalent interactions between the protein surface and the surface of the MCF. Affected by the pore walls enzyme immobilization in general can lead to a higher stability compared to the free enzyme.[2]
Not only the reusability of immobilized enzymes is important for upscale processes or industrial applications but also a recycling of the cofactors is desirable. Here we present an enzyme cascade using immobilized enzymes, employing the nicotinamide adenine dinucleotide phosphate ($NADP^+$) dependent glucose-6-phosphate dehydrogenase (G6PDH), 6-phospho–gluconate dehydrogenase (6PGDH) and alcohol dehydrogenase (ADH). G6PDH catalyzes the oxidation of glucose-6-phoshate (G6P) to give 6-phosphoglucono-δ-lactone (6PGL) which immediately hydrolyzes to 6-phosphogluconate (6PG), whereas 6PGDH enables the oxidation of 6PG to ribulose-5-phosphate (Ru5P). Both enzymatic reaction steps are accompanied by the reduction of an equivalent $NADP^+$ to NADPH, respectively.[4] $NADP^+$ can be regenerated from NADPH due to an ADH-catalyzed oxidation with concomitant reduction of 6-methyl-5-hepten-2-one (sulcatone) to 6-methyl-5-hepten-2-ol (sulcatol) (Figure 1).[5]

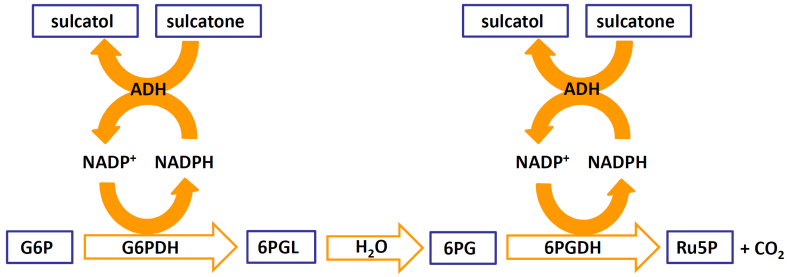

Figure 1. Enzymatic cascade reaction employing a reaction sequence of the pentose phosphate pathway: G6PDH and 6PGDH generate NADPH, whereas ADH catalyzes the oxidation of NADPH to $NADP^+$.

Related to the respective protein sizes we tailored the synthesis of MCF to reach pore sizes of 18.5 nm (windows) and 30.8 nm (cells) respectively. As qualified support material for the immobilization of G6PDH aminopropyl functionalized MCF has established.[2] To initiate attractive interactions between the surface of 6PGDH as well as ADH and the silica host material, the pore walls of MCF have been functionalized with alkyl and aminoalkyl residues consisting of different chain lengths (-C_3, -C_5, -C_7, -C_{11}, and -C_3NH_2, -C_5NH_2, -C_7NH_2, -$C_{11}NH_2$). Enzyme uptakes, loading densities, longtime stabilities and Michaelis-Menten kinetics of the enzymes immobilized onto the above-named silicas were investigated. Furthermore, each modified MCF has been analyzed by nitrogen physisorption, thermal analysis and zeta potential titrations.

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