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Immobilization of formaldehyde dehydrogenase in tailored siliceous mesostructured cellular foams and evaluation of its activity for conversion of formate to formaldehyde

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ABSTRACT

Formaldehyde dehydrogenase (FaldDH) is used as a catalyst to reduce formate to formaldehyde in a cascade reaction to convert CO_2 to methanol. This enzyme, however, has low activity and is sensitive to substrate/product concentration and pH. To improve the performance of FaldDH, it can be immobilized through physical adsorption in siliceous mesostructured cellular foams (MCF), which physical properties are suitable for the immobilization of large molecules as FaldDH (molecular size of 8.6 × 8.6 × 19 nm). In this work two MCF materials were synthesized: MCF1 with a pore size of 26.8 nm and window size of 10.5 nm; and MCF2 with a pore size of 32.9 nm and window size of 13.0 nm. The surfaces of the materials were functionalized with octyl, mercaptopropyl or chloromethyl groups. FaldDH was successfully immobilized inside all the materials, yielding enzyme loadings of about 300 mg g^{-1} in MCF1 and more than 750 mg g^{-1} in MCF2. However, the enzyme was inactive upon immobilization on MCF1, whereas on MCF2 the enzyme retained its catalytic activity presumably owing to the larger pores of this material and the need for the enzyme to undergo configurational changes during the reaction. Using MCF2 functionalized with mercaptopropyl groups the activity of FaldDH was enhanced beyond that of the free enzyme. Additionally, low leakage of the enzyme from the MCF2 was observed during the reactions. Thus, tailored MCF is a highly attractive material for employment of the FaldDH enzyme.

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1. Introduction

Formaldehyde dehydrogenase (FaldDH) catalyses the reversible reduction of formate to formaldehyde, using the cofactor nicotinamide adenine dinucleotide (NADH) as the terminal electron donor. This is a very relevant reaction for the multi-enzymatic bioconversion of CO_2 to methanol [1,2]. In this bioprocess, formate dehydrogenase (FateDH) converts CO_2 to formic acid, which is reduced to formaldehyde by FaldDH, and in the final reaction formaldehyde is reduced to methanol by alcohol dehydrogenase (ADH) [1]. Recent studies have shown that the intermediate step using FaldDH is the bottleneck reaction, since this enzyme shows low activity and is sensitive to substrate/product concentration and pH [2,3]. Therefore, it is necessary to investigate new ways to improve the stability, activity and applicability of this enzyme as a reducing agent. The immobilization of FaldDH in a porous host is a possible alternative to overcome these problems. Enzymes immobilized inside of porous materials become more stable due to the many points of interactions with the support and immobilization may also lead to higher activity [4]. FaldDH has been previously immobilized aiming at improved enzymatic activity for the oxidation of formaldehyde to formate (the reverse reaction to the one in this study) [5]. However, to the best of our knowledge, no material has been specifically tailored for the immobilization of FaldDH aiming at its improvement as a reduction catalyst for the conversion of formate to formaldehyde.

Enzymes can be immobilized in porous materials through physical adsorption [6–10]. In this method, the interactions between the support and the enzyme occur by van de Waals forces, hydrogen bonding, hydrophobic- and/or electrostatic interaction [4,11]. Since it does not involve any chemical modification of the enzyme it does not require use of any special equipment or conditions (e.g. low or high pH, organic solvents) and is considered a simple and inexpensive technique for enzyme immobilization [4,10,12–15]. Among the inorganic porous materials that can be used for the physical adsorption of enzymes, mesoporous silica has shown to be advantageous [16,17]. These materials can be synthesized with diverse pore structures (e.g. cubic, hexagonal, foam like) and tunable pore size (from 2 to 50 nm) providing an opportunity for selection of hosts

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that are optimal for the immobilization of each specific enzyme [18,19]. The structure of the pore is an important factor to be taken into consideration when developing suitable materials for enzyme immobilization. Choosing a structure with easily accessible pores, allowing for easy diffusion and avoiding pore blocking, usually leads to higher enzyme loading and fast adsorption kinetics [4,20,21]. The pore size is obviously also highly relevant and it should be tuned to be slightly bigger than the enzyme. If the pores are too big, the enzymes tend to become unstable inside of the pores and leach out more easily [4,10,14,15].

Since FaldDH is a large prolate-shaped enzyme, with molecular size of 8.6 nm × 8.6 nm × 19 nm [22], a host material with large mesopores is required. Such large mesopores are found in siliceous mesostructured cellular foams (MCF). These materials have large pores varying from 20 to 40 nm in diameter connected via smaller windows (of about 10–20 nm) in a structure that resembles aerogels [19]. It is thus likely that the structure of MCF provides an environment where the FaldDH may have a certain degree of motion inside of the pores, but cannot easily leach out due to the smaller windows. The high porosity of the MCFs is expected to also facilitate the diffusion of substrate and products that become more accessible to the immobilized enzyme.

To further improve MCF as a support carrier for FaldDH, the surface of the MCF can be modified with functional groups [23–25]. The presence of some specific organic groups in the silica surface changes the type of interaction involved in the physical adsorption of the enzyme in the support, which in principal can result in higher adsorption stability (lower leakage) and also can change the orientation of the enzymes inside the pores in a way that the active site is more accessible to the substrate molecules [15,23]. Due to the fact that FaldDH has a very complex structure [22] (formed by a homotetramer of identical subunits, each of them with 398 amino acid residues and 2 zinc ions) it is not trivial to predict how the chemical properties of the MCF surface should be adjusted for this enzyme to achieve optimal interactions. Hence, in order to find the optimized properties of the MCF for the immobilization of FaldDH, we have in this work prepared two MCFs with different pore and window sizes and functionalized their surfaces with octyl, chloromethyl or mercaptopropyl groups. The effects of the different physical and chemical properties of MCFs in the immobilization of FaldDH were evaluated in terms of enzyme loading capability and enzyme activity.

2. Materials and methods

2.1. Materials and chemicals

Triblock copolymer pluronic™ P123 ($EO_{20}PO_{70}EO_{20}$, Mw = 5800), 1,3,5 trimethylbenzene (TMB, 98%), tetraethylorthosilicate (TEOS, ≥98%), hydrochloric acid (HCl) (37 wt%), ammonium fluoride (NH_4F, ≥99,9%), chloromethyltriethoxysilane (CMTS, 96%), octyltriethoxysilane (OCTS, ≥97.5%), 3-mercaptopropyltrimethoxysilane (MPTS, 95%), toluene (anhydrous, 99.8%), potassium phosphate monobasic (KH_2PO_4, ≥98%), potassium phosphate dibasic (K_2HPO_4, ≥98%) β-nicotinamide adenine dinucleotide reduced disodium salt hydrate (NADH, 98%), sodium formate ($CHNaO_2$, ≥99%) and formaldehyde dehydrogenase from *Pseudomonas* sp. (1.0-6.0 units/mg solid) obtained as lyophilized powder were all purchased from Sigma-Aldrich.

2.2. Methods

2.2.1. Synthesis of siliceous mesostructured cellular foams

Two siliceous mesostructured cellular foams (MCF1 and MCF2) were synthesized according to the protocol adapted from Schmidt-Winkel et al. [19] and Sridhar et al. [26], where TEOS is the silica precursor, the triblock copolymer pluronic™ P123 is used as structure direct agent, TMB acts as organic swelling agent and NH_4F is used to enlarge the windows between the pores. The procedure for the synthesis of MCF1 has been reported previously [27]. For MCF2 a similar method was used, but the mass ratio of TEOS to surfactant, and TMB to surfactant were changed from TMB/P123 = 1.5 and TEOS/P123 = 2.2 for MCF1 to TMB/P123 = 2.0 and TEOS/P123 = 4.2 for MCF2. The surface of the MCF1 and MCF2 were functionalized with chloromethyl (CM), octyl (OC) or mercaptopropyl (MP) groups using a similar procedure as presented by Russo et al. [23]. The functionalized MCFs were named as MCF(1or2)-XX, where "XX" corresponds to the functional group in the surface (CM, OC, MP). See supplementary information for a detailed description of the synthesis and functionalization of MCF.

2.2.2. Characterization of the MCFs

The physical properties of the MCFs were determined by nitrogen sorption analysis, using a TriStar 3000 instrument from Micromeritics Instrument Corporation. Prior to the adsorption measurements, the non-functionalized MCFs were outgassed for at least 8 h at 180 °C whereas the functionalized MCFs were outgassed overnight at 120 °C. The simplified BdB(Broekhoff – deBoer)-FHH(Frenkel-Halsey-Hill) method [28] was used to calculate the pore/window size distribution and the BET (Brunhauer-Emmett-Teller) method was applied to obtain the specific surface area. The specific pore volume was calculated using a single point adsorption value at the relative pressure of 0.990.

The particle size and morphology of the MCF was determined from scanning electron microscopy using an Environmental SEM, FEI Quanta 200 FEG, operating at 5 kV or 10 kV.

The functionalized MCFs were also characterized by thermogravimetric analysis (TGA) using a Mettler Toledo – TGA/DSC 3+, with a heating rate of 10 °C min^{-1} under a N_2 flow of 50 ml min^{-1}. From the weight losses obtained by heating from 200 °C to 750 °C, the surface loading (N_S) [29] and surface density (D) [30] were calculated for each functionalized MCF using Eqs. (1) and (2), respectively. "W_{loss}" is the weight loss, "N_A" is Avogadro's number, "M_W" is the molecular weight of the ligand and "SA" is the specific surface area of the MCF.

$$N_S = W_{loss}/(100gMCF \cdot M_W\ ligand) \quad (1)$$

$$D = N_A \cdot N_S/SA \quad (2)$$

2.2.3. Immobilization of FaldDH in MCFs

0.1 g of each MCF were dispersed in 5 ml of phosphate buffer at pH 5.6 and sonicated for 10 min to prevent particle agglomeration. After that, 100 µl of the MCF suspensions were added to 200 µl of FaldDH with different concentrations (2, 6 and 10 mg ml^{-1}) until the maximum amount of enzymes were immobilized. The immobilization was performed at pH 5.6, which is close to the pI of the enzyme (pI = 5.3) to reduce enzyme–enzyme repulsive interactions. The immobilization in MCF1 was also evaluated at pH 7.0 to investigate the influence of the pH in the enzyme immobilization. The samples were placed in a thermomixer for 24 h at 37 °C and 300 rpm. After that, the samples were centrifuged and washed with phosphate buffer at pH 6.5 two times. The amount of enzyme immobilized was estimated by measuring the residual protein concentration in the supernatant by UV absorbance at 280 nm (extinction coefficient: $E^{1\%} = 10.0$) using the Nanodrop One instrument from Thermo scientific. The experiments were performed in triplicate.

The pore loadings (PLD) and pore fillings (Pf) were calculated according to Eqs. (3) and (4) [17], respectively, where "W_{enz}" is the total amount of enzyme immobilized, "W_{MCF}" is the weight of MCF used for the immobilization, "V_{enz}" is the volume of the enzyme,

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