Catalysis Today 157 (2010) 94–100

Contents lists available at ScienceDirect

Catalysis Today

journal homepage: www.elsevier.com/locate/cattod

Oxidation reactions using air as oxidant thanks to silica nanoreactors containing GOx/peroxidases bienzymatic systems

P. Laveille, L. Truong Phuoc, J. Drone, F. Fajula, G. Renard, A. Galarneau*

Institut Charles Gerhardt Montpellier, UMR 5253 CNRS/UM2/ENSCM/UM1, ENSCM, 8 rue de l'Ecole Normale, 34296 Montpellier Cedex 5, France

ARTICLE INFO

Article history:
Available online 2 April 2010

Keywords:
Biocatalysis
Immobilized enzymes
Mesoporous silica
Phospholipids
Hemoglobin
Glucose oxidase

ABSTRACT

A new kind of enzymes encapsulation method is presented. It combines the sol–gel method with a templating process using bilayers of phospholipids to provide an organized network of phospholipids inside the silica and in the same time protect the embedded enzymes, as if they were entrapped in a biological membrane supported on silica. This brings a porosity control to the classical sol–gel encapsulation and therefore increases the accessibility of the substrates to a maximum of enzymes. A first family of hybrid phospholipids–silica materials, named Sponge mesoporous silica (SMS), has been synthesized, which features a sponge-like structure. The formation of a negative curvature in the rigid phospholipid bilayers was induced by adding ethanol (>50%) to the aqueous medium, which provokes the separation of the head groups and the undulations of the lipid bilayers. Dodecylamine added to stabilize this structure acts as a catalyst for silica condensation. Lactose was added to avoid direct interaction of the enzymes with the silica at the interface with the lipids bilayer. Lipase was encapsulated in SMS and shows higher activity for ester hydrolysis compared to commercial immobilized-lipase. An experiment design plan was carried out to optimize SMS synthesis using hemoglobin (Hb) as biocatalyst for oxidation reactions using H_2O_2. As a result, a new structure was identified, formed by the aggregation of nanoporous silica capsules, named NPS, with a higher activity compared to SMS biocatalysts. These new biological nanoreactors were used to encapsulate simulteanously Hb and glucose oxidase, to produce in situ H_2O_2 from air, and perform oxidation reactions. This system was used for the elimination of the carcinogenic polycyclic aromatic hydrocarbon pollutants from water.

1. Introduction

Enzymes are very selective catalysts and may represent a suitable response for the challenges of the 21st century in catalysis with respect to improvement of the selectivity of the reactions. For an industrial application, a challenge is to find a way to increase the stability of enzymes towards catalytic inactivation by temperature, inhibitors, mechanical treatments, solvents or pH variations and to recover the biocatalyst from the medium after reaction. The solution is to heterogenize the biocatalyst by immobilizing it in an inorganic matrix without deactivating it (Fig. 1). More and more studies have selected silica as inorganic supports [1–4] and have shown that mesoporous silicas are very efficient supports to maintain the activity of the biomolecules [5–7]. Different methods can be used to immobilize enzymes in inorganic supports such as covalent binding, adsorption and sol–gel encapsulation (Fig. 1) [8–11]. All of these methods have advantages and disadvantages. The adsorption of enzymes in an inorganic support is the cheapest and the easiest, but is often susceptible to a progressive leaching. The grafting of enzymes into a support avoid the leaching, but is more time consuming as it implies a previous functionalization of the support before to react with a part of the protein, which can sometimes lead to the denaturation of proteins. Silica sol–gel entrapment represents a good compromise. However, the direct interaction of proteins with silanols should be minimized to avoid protein deformation, and, soluble excipients are added to stabilize the correctly folded protein conformation. Additives help also to stabilize proteins against the denaturing stresses encountered upon sol–gel entrapment. Silica sol–gel has allowed to maintain activity of enzymes [12] or bacteria [13–15] by using additives such as sugars, glycerol, charged polymers (poly-vinylimidazole, -ethyleneimine, -ethyleneglycol) or gelatin. For lipases encapsulated in sol–gel, poly(vinyl alcohol) [16,17] has been used and the synthesis has been further developed commercially by Fluka. Nevertheless, in sol–gel encapsulation, the lack of controlled porosity limits the diffusivity of the substrates. It is why we have developed a new concept of sol–gel synthesis [9,10]. It combines the sol–gel method with a templating process using bilayers of phospholipids to provide an organized network of phospholipids inside the silica and in the same time protect the embedded enzymes

* Corresponding author.
E-mail address: anne.galarneau@enscm.fr (A. Galarneau).

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doi:10.1016/j.cattod.2010.02.065

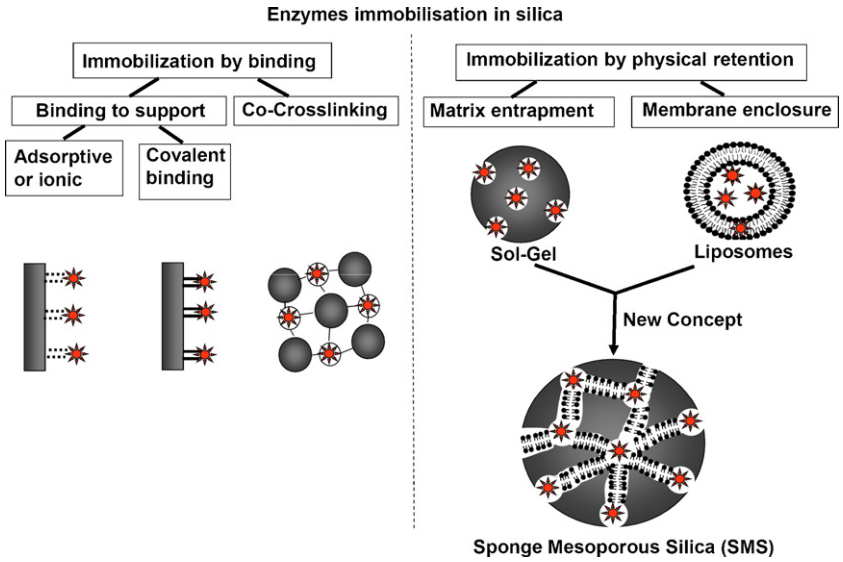

Fig. 1. Schematic representation of the different ways to immobilize enzyme.

(Fig. 1), as if they were entrapped in a biological membrane supported on silica. This brings a porosity control to the classical sol–gel encapsulation and therefore increases the accessibility of the substrates to a maximum of enzymes. Different structures of biomaterials have been obtained and have been used for ester hydrolysis and oxidation reactions with lipase and hemoglobin (Hb), respectively. A bienzymatic system composed of glucose oxidase and Hb allows to perform oxidation reactions from air by producing in situ H_2O_2. The removal of carcinogenic polycyclic aromatic hydrocarbon (PAH) pollutants from water has been realized thanks to this new nanobioreactor.

2. Experimental

2.1. Materials

Sponge mesoporous silica (SMS) and nanoporous silica capsules (NPS) have been synthesized using tetraethoxysilane (TEOS) from Aldrich, lecithin from egg yolk (L-α Lecithin) (Fluka) and β-D-lactose (Aldrich). Egg yolk lecithin is a natural and low cost phospholipid. Lipase from *Mucor Meihei* and glucose oxidase from *Aspergillus Niger* were purchased from Fluka. Bovine methemoglobin (Hb) was obtained from Sigma–Aldrich (ref H2625). H_2O_2 was obtained as a 35% solution from Sigma–Aldrich. Chemicals for the buffers were reagent grade (Fluka). PAHs (naphthalene, acenaphtene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, benzo(g,h,i)perylene, indeno(1,2,3,cd)pyrene) and 2,2′-azinobis(3-ethylbenzthiazoline-6-sulphonic acid) diammonuim salt (ABTS) were purchased from Sigma–Aldrich (98–99.8% purity). Acetonitrile was of HPLC grade (SDS).

2.2. Sponge mesoporous silica (SMS) synthesis

SMS synthesis was performed by using the following reactants: TEOS, dodecylamine, lecithin, β-D-lactose. For a typical SMS synthesis, the molar ratios used were: 1 TEOS/0.11 lecithin/0.08 dodecylamine/0.068 lactose/56 H2O/17 ethanol. A first solution of 0.15 g lactose and 7 mL phosphate buffer (pH 5 for Hb and GOx, and pH 8 for lipase), containing the desired amount of enzyme (300 mg Hb, 50 mg GOx, 10 mg lipase/g TEOS), was prepared at 37 °C and added slowly under vigorous stirring to a second solution, prepared at 37 °C, containing 0.5 g lecithin and 0.1 g dodecylamine in 5.2 g ethanol, until a homogeneous emulsion was formed. 1.35 g TEOS was then added slowly under vigorous stirring for 15 min, and stir until a homogeneous emulsion was formed, then the mixture was left for 24 h in static conditions at 37 °C. The resulting powder was then centrifuged and washed 5 times with 10 mL of phosphate buffer followed by centrifugation. The samples were then dried by lyophilization.

2.3. Synthesis of nanoporous silica capsules (NPS)

NPS synthesis uses the same reactants as for SMS synthesis, with different molar ratios. For a typical NPS (NPS6), the molar ratios used were: 1 TEOS/0.2 lecithin/0.06 dodecylamine/0.03 lactose/83 H2O/9 ethanol, which corresponds to 0.05 g lactose, 7.2 mL phosphate buffer containing the desired amount of enzyme, 0.7 g lecithin, 0.05 g dodecylamine, 2 g ethanol and 1 g TEOS.

2.4. ABTS activity

The ABTS reaction is considered as a model reaction for the peroxidase activity of enzymes [18]. For the evaluation of the immobilized-Hb biomaterials, a home-made continuous flow cell maintained at 25 °C, consisting of a peristaltic pump, a mechanical stirrer, and a bottom opened flask equipped by a fritted glass was used. Typically, 19.6 mL of phosphate buffer pH = 6 (I = 50 mM) are loaded to the flask together with 700 μL of ABTS solution at a concentration of 75 mM (50 μmol ABTS) and 10–20 mg of Hb-biomaterial. The pump and the stirrer are activated and the reaction starts by adding 700 μL of H_2O_2 at a concentration of 75 mM (50 μmol H_2O_2). The formation of the colored product ($ABTS^+$, oxidized ABTS) is followed at 414 nm with an UVIKON 930 spectrophotometer from Kontron Instrument polystat 22/86602 Bioblock integration software. Activity of free Hb was 1015 μmol/min/g Hb.

2.5. PAH degradation with NPS nanobioreactors

Studies of PAH oxidation were conducted in a 100 mL reaction mixture containing 6 g/L Hb/GOx–NPS (or 0.8 g/L GOx–NPS and 6 g/L Hb–NPS), 300 nM of each PAH, citrate buffer pH = 5 (I = 50 mM), 150 mM glucose and 1% (v/v) acetonitrile. The NPS synthesis corresponds to the experiment 6 in the experiment design plan. The kinetic of the PAH oxidation was followed by ultrafast liquid chro-

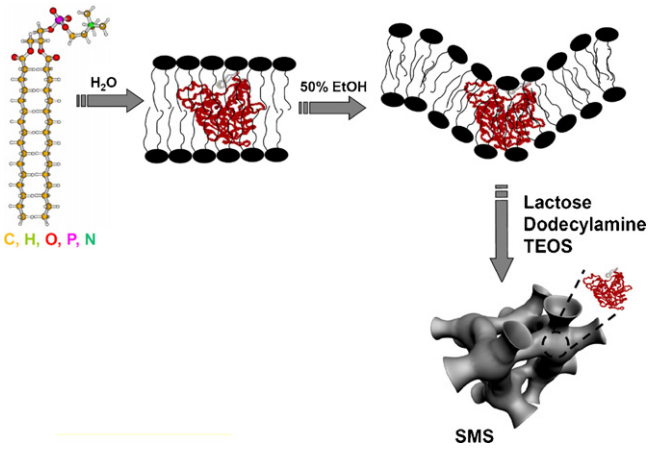

Fig. 2. Schematic representation of sponge mesoporous silica (SMS) formation, from phospholipids and lipids bilayer undulation.

matography (UFLC). PAH degradation was calculated from the decreasing area of chromatogram peaks between reaction time $t = 0$ and $t = x$ min. Results of total degradation of PAH were taken after 2 h. With this method, the detection limit is 15 ± 7.5 nM. Reactions were performed in flasks protected by aluminum foil to avoid PAH photo-oxidation cross-reaction. To ensure reproducible results, after reaction and before UFLC analysis, samples have been diluted in 50% (v/v) acetonitrile and filtrated on a regenerated cellulose membrane syringe filter from Macherey-Nagel (25 mm, 0.2 μm pore diameters) to eliminate solid particles and to stop the reaction. Blank reactions without glucose have also been performed to estimate the proportion of PAH adsorbed on the biomaterials.

2.6. Characterization

Powder X-ray diffraction (XRD) patterns were recorded on a Bruker AXS D8 diffractometer by using Cu Kα radiation and Ni filter. The adsorption–desorption isotherms for nitrogen at 77 K were measured using a Micromeritics ASAP 2010 instrument on samples previously calcined at 550 °C in air for 8 h to study the porosity of the material after removal of phospholipids and enzymes. Examination of the particles morphology was achieved using a Hitachi S-4500 I scanning electron microscope (SEM). Transmission electron microscopy (TEM) was performed on a JEOL 1200 EX II microscope operating at 120 keV. The particles were trapped in a resin (LR White) and cut into slices 70 nm thick by ultramicrotomy before to be imaged.

3. Results and discussion

3.1. Sponge mesoporous silica (SMS) encapsulation method

"Sponge mesoporous silica" (SMS) are synthesized using lecithin/dodecylamine/lactose as templates in an ethanol/aqueous media [9,10–19,20], and are suitable for enzyme encapsulation. Lecithin belongs to the phospholipids family, which forms the lipid matrix of biological membranes. As a biocompatible surfactant, it is widely used in every day life, including human and animal food, medicine, cosmetics and manifold industrial applications [21]. Because of its nearly cylindrical molecular shape, lecithin cannot grow micelles by itself in aqueous media. Its tendency to curve, described in terms of its spontaneous curvature, is very low, and therefore it induces the formation of lamellar bilayer phospholipids structures, described as membranes, vesicles or liposomes. The effect of the addition of ethanol into biological membranes in aqueous solution is a subject of debate and controversy. A very recent study of structure simulation concerning the interaction of

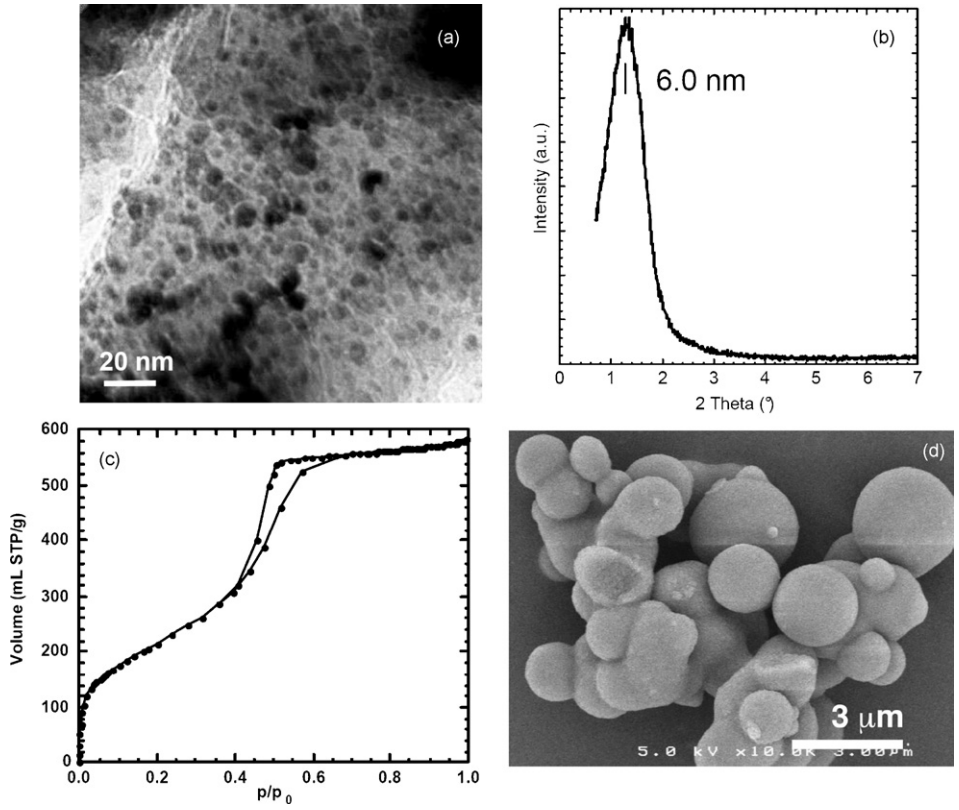

Fig. 3. (a) TEM, (b) XRD, (d) SEM of as-synthesized SMS and (c) nitrogen sorption at 77 K of calcined SMS.

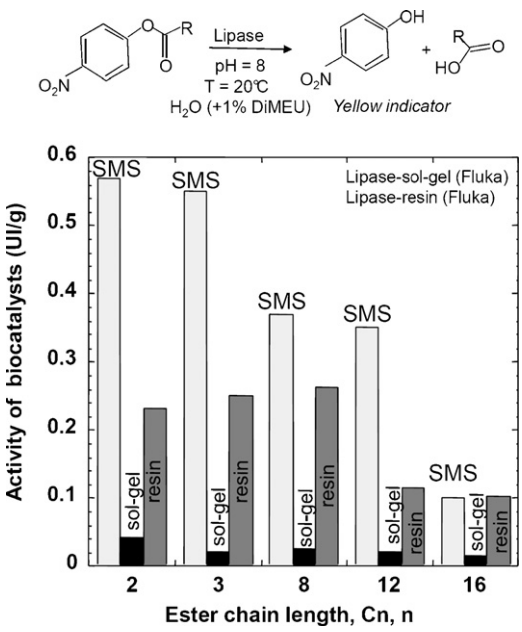

Fig. 4. Activity towards ester hydrolysis per g of biomaterials of lipase immobilized in SMS, in sol–gel or in resin, in function of ester chain length.

ethanol with phospholipid bilayers helps to understand the effect of ethanol on bilayer structures [22]. The addition of ethanol gives rise to an increase in the head group area leading to: (i) a disordering of the lipid acyl chains; (ii) a diminution of the bilayer thickness by interdigitation of the lipid chain; and (iii) an expansion of the membrane, creating a reduction of its rigidity and an increase of its fluidity, responsible of an enhanced permeability. At high ethanol content (50% in volume), ethanol molecules penetrates into the hydrophobic core of the bilayer increasing the hydrophilicity of the membrane interior, and a wave motion (Fig. 2) of the lipid bilayer arises, creating positive and negative curvatures in the membrane. The insertion of dodecylamine into the phospholipids/EtOH/H_2O system stabilizes the structure of the resulting mesophase by maintaining the space between lipid heads. In the same time, dodecylamine catalyzes the polymerization of the alkoxyde of silica (TEOS) around this mesophase to lead to the SMS materials. Lactose is present at the interface between the membrane and the silica to minimize the direct interaction of the enzyme with silica. Resulting SMS materials (Fig. 3) show a three-dimensional "sponge mesoporous" porosity with a pore diameter of about 5–6 nm, in accordance to the length of a lecithin bilayer, evidenced by XRD and by nitrogen adsorption of calcined SMS. Surface area and pore volume of calcined SMS increases with the amount of dodecylamine used in the synthesis from 500 to 700 m^2/g and from 0.4 to 0.7 mL/g for dodecylamine/TEOS molar ratio of 0.05–0.13, respectively [20]. SMS features spherical particles of 3 μm as observed in SEM images (Fig. 3).

In the presence of lipase from *Mucor miehei* during the synthesis, the lipase-SMS exhibited higher catalytic activity per g of biocatalyst (Fig. 4), compared to the same lipase immobilized in commercially available supports: lipase encapsulated in silica sol–gel (Fluka) or post-synthesis resin immobilization ("Lipozyme"-Fluka) [9,10]. The highest efficiency of SMS encapsulation was calculated by comparing the relative activity of the immobilized enzyme to the activity of the free enzyme and was of 154% for lipase-SMS and 4% lipase-sol–gel for the hydrolysis of p-nitrophenol propionate. This calculation was done thanks to the dissolution of the silica matrix in basic solution and by measuring the amount of resulting protein by the Bradford technique. Unfortunately this dissolution method was unsuccessful for resin

Table 1
Experiment design plan to optimize SMS synthesis. For 7.2 mL phosphate buffer pH 6, the studied compositions are: Hb (−1) = 50 mg, (+1) = 300 mg; lactose (La) (−1) = 50 mg, (+1) = 300 mg; lecithin (Lec) (−1) = 300 mg, (+1) = 700 mg; dodecylamine ($C_{12}NH_2$) (−1) = 50 mg, (+1) = 150 mg; ethanol (−1) = 2 g, (+1) = 10 g; TEOS (−1) = 1 g, (+1) = 3 g.

N	Hb	La	Lec	$C_{12}NH_2$	EtOH	TEOS
1	−1	−1	−1	−1	−1	−1
	1	−1	−1	−1	−1	1
3	−1	1	−1	−1	−1	1
4	1	1	−1	−1	−1	−1
5	−1	−1	1	−1	−1	1
6	1	−1	1	−1	−1	−1
7	−1	1	1	−1	−1	−1
8	1	1	1	−1	−1	1
9	−1	−1	−1	1	−1	1
10	1	−1	−1	1	−1	−1
11	−1	1	−1	1	−1	−1
12	1	1	−1	1	−1	1
13	−1	−1	1	1	−1	−1
14	1	−1	1	1	−1	1
15	−1	1	1	1	−1	1
16	1	1	1	1	−1	−1
17	−1	−1	−1	−1	1	1
18	1	−1	−1	−1	1	−1
19	−1	1	−1	−1	1	−1
20	1	1	−1	−1	1	1
21	−1	−1	1	−1	1	−1
22	1	−1	1	−1	1	1
23	−1	1	1	−1	1	1
24	1	1	1	−1	1	−1
25	−1	−1	−1	1	1	−1
26	1	−1	−1	1	1	1
27	−1	1	−1	1	1	1
28	1	1	−1	1	1	−1
29	−1	−1	1	1	1	1
30	1	−1	1	1	1	−1
31	−1	1	1	1	1	−1
32	1	1	1	1	1	1

immobilization and the amount of enzyme is not given by the supplier, so its efficiency is unknown. However in real catalytic application, the activity per g of biocatalyst is the most relevant data and lipase-SMS is the most active. It was demonstrated that lactose was the more efficient sugar to preserve enzyme-SMS activity. The high catalytic activity was explained by the use of lecithin/dodecylamine assembly structure permitting facile diffusion of substrates, through the generation of a 3D mesoporosity in silica sol–gel, and also to the use of natural and biocompatible surfactants. However, a change in typoselectivity of the enzyme was noticed for lipase encapsulated in SMS materials. Indeed, whereas the free lipase hydrolyzes preferentially long chain esters with the highest selectivity for chains with 8 carbons, the lipase-SMS exhibits the highest selectivity for shorter chains with 2 or 3 carbons, that free lipases are not able to hydrolyze. A change in the conformation of the lipase has occurred during the SMS encapsulation changing the selectivity of the enzyme.

3.2. Nanoporous silica capsules (NPS) for enzyme encapsulation

In order to increase the performance of SMS materials, an experiment design plan of 32 experiments (Table 1) has been performed by varying the reactants molar ratio and using hemoglobin (Hb) as active biocatalyst for oxidation reactions in presence of H_2O_2 [23–25]. The ABTS oxidation test [18] was performed to evaluate rapidly the activity of the biomaterials. In Fig. 5, only the results for the higher amount of Hb (300 mg Hb/g TEOS) in the biomaterials have been presented for more clarity, as biomaterials prepared with the lower amount of Hb (50 mg/g TEOS) features much lower activity. From the experiment design plan, one can calculate the favorable factors responsible for a higher activity of

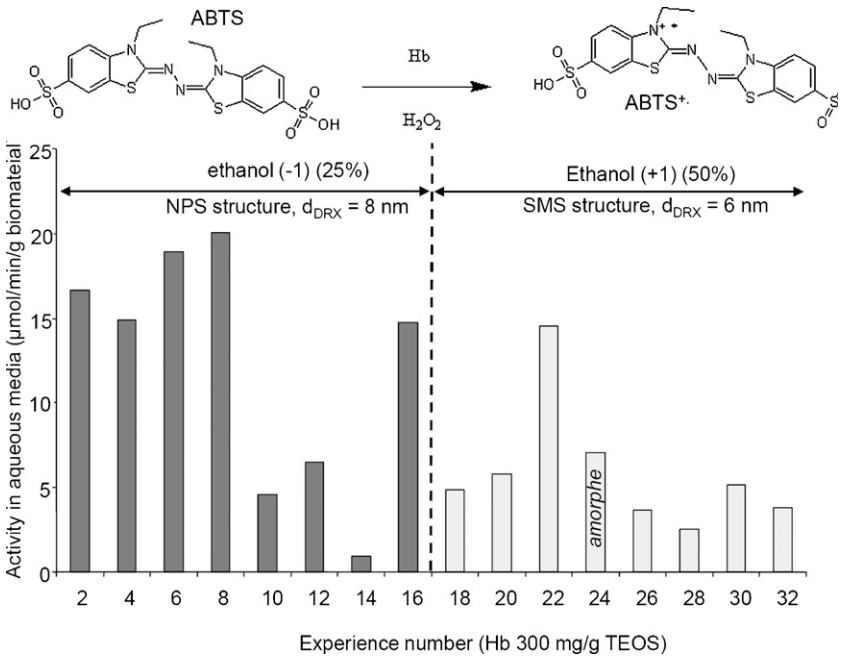

Fig. 5. ABTS activities per g of biomaterials of encapsulated hemoglobin synthesized from the experiment design plan described in Table 1 (for 300 mg Hb/g TEOS).

the biomaterials: Hb (+4.2) > lecithin (+0.9) > lactose (+0.3), and the unfavorable factors: dodecylamine (−2.17) > ethanol (−1.8) > TEOS (−0.2). Unfortunately, this experiment design plan could not be totally exploited as a new material family, named nanoporous silica capsules (NPS), was formed at lower amount of ethanol (25% in volume) characterized by a larger *d*-spacing (d_{XRD} = 8 nm) than SMS materials (d_{XRD} = 6 nm). Most of the members of this new NPS family (as the one coming from experiments number 2, 4, 6, 8) exhibits a higher activity than SMS biomaterials. The characterization of the NPS resulting from experiment number 6 (named NPS6) shows that NPS biomaterials are built of silica nanocapsules of 7 nm aggregated in larger cells of 20 nm as raspberries. Each nanocapsule is connected to the others by an opening of around 1 nm (Fig. 6). The SEM morphology of the NPS materials is totally different of the one of SMS materials, as the NPSs feature an aggregation of nanometric particles and SMSs present large sphere of 2–3 µm. After

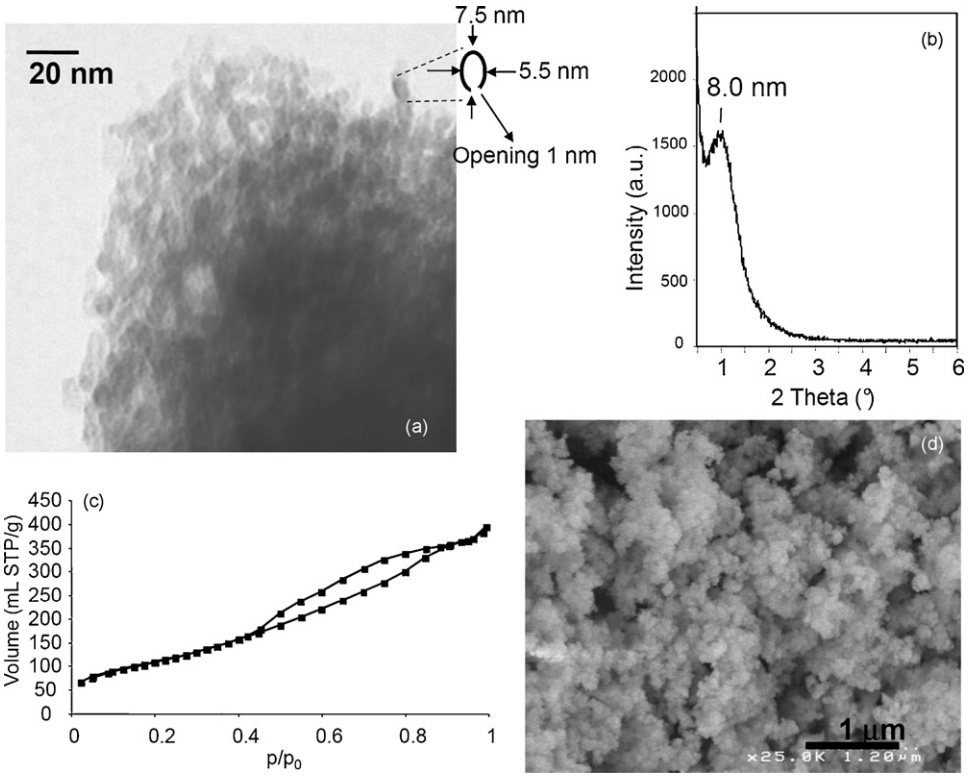

Fig. 6. (a) TEM, (b) XRD, (d) SEM of as-synthesized NPS6 and (c) nitrogen sorption at 77 K of calcined NPS6.

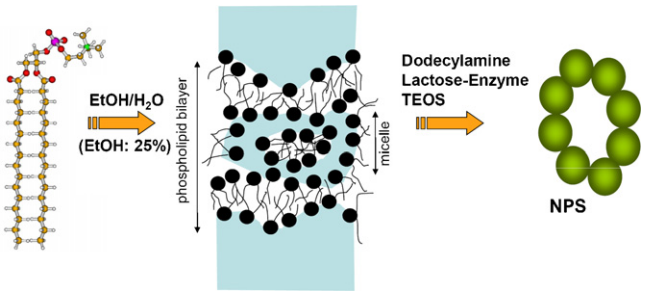

Fig. 7. Schematic representation of porous nanoporous silica capsules (NPS) formation, from phospholipids and formation of micelles of phospholipids inside lipids bilayer.

calcination, the XRD peak of NPS disappears; NPS structure is not stable upon calcination in contrary to SMS materials. By nitrogen adsorption only some residual porosity can be detected. The formation of NPS materials is explained by the feasibility of micelles of phospholipids when lower ethanol amount and larger amount of phospholipids are used in comparison to SMS materials. In simulation study [22], it has been shown that ethanol facilitate the water penetration in the membrane interior as well as the migration of phospholipids, giving rise to clusters of water molecules (in blue in Fig. 7) surrounded by lipids head groups in the membrane interior, forming micelles of 7 nm large and 4 nm high inside the undulated bilayer of 7 nm high, with possible connections between the micelles and the bilayer. The formation of these micelle-like structures is on line with our TEM observation of NPS materials, where silica takes the place of the water in the interior of the bilayer and polymerize around the micelles of phospholipids, which are then expulsed from the distorted membrane.

3.3. NPS as nanobioreactors for oxidation reaction from air

The choice of the biocatalyst for an oxidation reaction is crucial and depends on its activity, stability, and its economic impact on the process [26]. Enzymes such as oxygenases and oxidases (P_{450} cytochromes [27] and laccases [28]), which directly use O_2 to oxidize their substrates, can be used. The disadvantage to this biotechnology is the high cost of the enzymes due to expensive extraction and purification processes that are needed. Bovine hemoglobin, a non-enzymatic member of the hemoprotein family, presents the advantage to be a low cost waste product of the food industry, and is able to perform oxidation reactions through a peroxidase-like pathway using H_2O_2 [23–25]. However, in industrial processes, H_2O_2 is difficult to handle and oxidation from air is preferred. In order to use directly air as oxidant, a bienzymatic system has been built thanks to another industrially used enzyme, the glucose oxidase (GOx), which produces in situ H_2O_2 from oxygen dissolved in water and glucose (Fig. 8). GOx and Hb have been encapsulated in the same NPS6 nanobioreactor with 50 mg GOx/g TEOS and different amount of Hb from 50 to 300 mg Hb/g TEOS. The activity of Hb/GOx–NPS towards ABTS oxidation has been compared to the direct addition of H_2O_2 to Hb–NPS (Fig. 8). Results show that the activity of the biomaterials increases linearly with the amount of Hb and that Hb/GOx–NPS nanobioreactors are less active than direct addition of H_2O_2 on Hb–NPS, but are enough efficient to perform oxidation reactions without adding any peroxides. The enzyme–NPS biocatalysts has been stored at 4 °C and after 60 days they show the same activity has the free enzyme in the same conditions (maintain 70% of activity). Reuse of enzyme–NPS has not been checked as we are working in batch condition with H_2O_2 (added or in situ produced), which is a suicide substrate able to destroy enzyme. Only continuous flow reaction could allow to test recyclability, but as NPS are nanoparticles, columns are impossible to fill, therefore only batch reactions are considered. In the following, the more active biomaterial (synthesized from NPS6 conditions with 50 mg GOx and 300 mg Hb/g TEOS) has been considered.

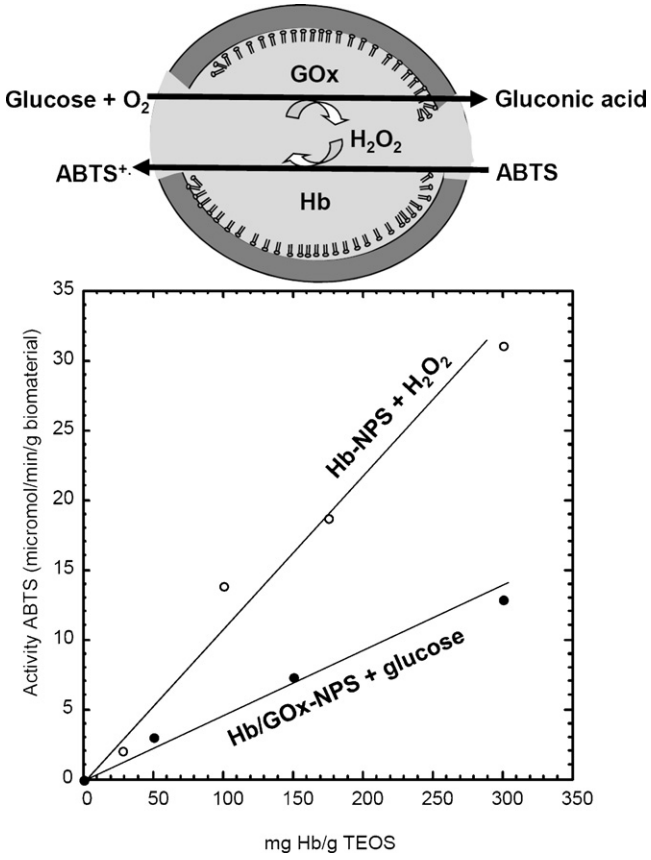

Fig. 8. ABTS activities per g of biomaterials for different amount of Hb encapsulated in NPS6 with addition of H_2O_2 or simultaneous with GOx (50 mg/g TEOS) with addition of glucose (without peroxide).

Recently, we have shown that free Hb was able to oxidize strong carcinogenic pollutants such as polycyclic aromatic hydrocarbon (PAH), such as anthracene (ANT), in water (with 1% acetonitrile) with a large excess of H_2O_2 and Hb compared to ANT, with molar ratios of $[H_2O_2]/[ANT] > 1000$ and $[Hb]/[ANT] > 1$ [25]. We have also shown that 65% of oxidized anthracene remained covalently bind to the protein [25]. Hb allows in the same time to oxidize and to fix the oxidation products. Heterogenizing Hb by NPS encapsulation will lead therefore to a direct removal of PAHs. In this study, in order to remove PAHs of waste water, such as in the waste water of oil refineries, Hb/GOx–NPS (6 g/L) was added to an aqueous solution containing 11 PAH (300 nM each) without any peroxides. Then 150 mM of glucose was added and let react for 2 h. The biomaterial is then removed by centrifugation and the solution is analyzed. All PAHs have reacted with Hb/GOx–NPS (Fig. 9). Some PAHs are more reactive than others as observed for anthracene and acenaphtene with 100 and 86% disappearance, respectively, and only 18 and 10% for phenanthrene and naphthalene. In total, more than 63% of PAH have disappeared from the water solution by this process. It is to notice that a blank test done without glucose addition shows that high molecular weight PAHs are strongly adsorbed in NPS materials, and that low molecular weight PAH, like anthracene and acenaphtene, are weakly adsorbed but strongly oxidized by the nanobioreactor. Hb/GOx–NPS oxidizes 80% of anthracene and 75% of acenaphtene in 2 h without adding any peroxides, whereas more than 20 h are needed for con-

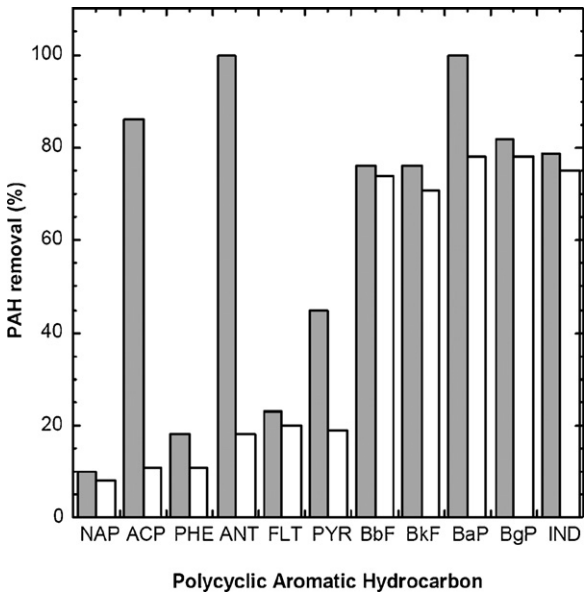

Fig. 9. (Grey bars) percentage of PAH removal from water using Hb/GOx–NPS6 nanobioreactor with glucose addition (without peroxide). (White bars) percentage of adsorbed PAH, the difference between grey and white bars are the percentage of oxidized PAH. List of PAHs: naphthalene (NAP), acenaphtene (ACE), phenanthrene (PHE), anthracene (ANT), fluoranthene (FLT), pyrene (PYR), benzo(b)fluoranthene (BbF), benzo(k)fluoranthene (BkF), benzo(a)pyrene (BaP), benzo(g,h,i)perylene (BgP), indeno(1,2,3,cd)pyrene (IND).

ventional heterogeneous catalysts like Ti–SiO_2 using peroxides to oxidize only anthracene, the more reactive PAH [29]. A comparison has been performed with a separate encapsulation of the two enzymes Hb and GOx to evaluate the efficiency of the proximity of the enzymes. A mixture of two NPS composed of 0.8 g/L GOx–NPS (50 mg GOx/g TEOS) and 6 g/L Hb–NPS (300 mg Hb/g TEOS) has been added to the water solution of the 11 PAHs. After addition of glucose, similar results (not shown) have been obtained for the (Hb–NPS + GOx–NPS) separated encapsulations compared to the (Hb/GOx–NPS) simultaneous encapsulation, with a slightly higher oxidation rate reaction for the biencapsulated Hb/GOx–NPS system. NPS encapsulation is therefore suitable for mono- and polyenzymatic systems and can perform oxidation reaction from air, when GOx is used to produce in situ H_2O_2.

4. Conclusion

"Sponge mesoporous silica" (SMS) and "nanoporous silica capsules" (NPS), synthesized using lecithin/dodecylamine/lactose as templates in an ethanol/aqueous media, are suitable for enzyme encapsulation. SMS and NPS encapsulation combines the sol–gel method with a templating process using bilayers of phospholipids to provide an organized network of phospholipids inside the silica and in the same time protect the embedded enzymes. Lipase-SMS biocatalysts exhibit a better activity for ester hydrolysis compared to commercial immobilized biocatalysts. Hb/GOx–NPS nanobioreactors were able to oxidize strong pollutants in water such as polycyclic aromatic hydrocarbon by simply adding glucose to the solution, without any use of peroxides. These new nanobioreactors bring the possibility to develop biotechnology for water depollution.

Acknowledgements

The authors are grateful for the financial support from the TOTAL France S.A company, and, thank Yann Ambacher and Gerard Baudouin from ENSCM for their participation in the experiment design plan.

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