Behavior Of Silica Aerogel Networks as Highly Porous Solid Solvent Media for Lipases in a Model Transesterification Reaction

H. El Rassy,*[a] A. Perrard,[a] and A. C. Pierre[a, b]

Highly porous silica aerogels with differing balances of hydrophobic and hydrophilic functionalities were studied as a new immobilization medium for enzymes. Two types of lipases from Candida rugosa *and* Burkholderia cepacia *were homogeneously dispersed in wet gel precursors before gelation. The materials obtained were compared in a simple model reaction: transesterification of vinyl laurate by 1-octanol. To allow a better comparison of the hydrophobic/hydrophilic action of the solid, very open aerogel networks with traditional organic hydrophobic/hydrophilic liquid solvents, this reaction was studied in mixtures containing different proportions of 2-methyl-2-butanol, isooctane, and water. The results are discussed in relation to the porous and hydrophobic nature of aerogels, characterized by nitrogen adsorption. It was found that silica aerogels can be considered as "solid" solvents for the enzymes, able to provide hydrophobic/hydrophilic characteristics different from those prevailing in the liquid surrounding the aerogels. A simple mechanism of action for these aerogel networks is proposed.*

KEYWORDS:

aerogels · enzymes · lipases · solvent effects · transesterification

Introduction

Recently, silica xerogels have been successfully developed as encapsulation media for lipases and applied to fatty component esterification, transesterification, or hydrolysis.[1–8] These biocatalysts are known, in the best cases, to operate more efficiently than the corresponding free enzymes.[5] However, their operating mechanism remains obscure.

It is known that the nature of the liquid solvent in which a lipase is dispersed, in particular its hydrophilic or hydrophobic character, is very important for control of the catalytic activity of the lipase.[9–14] Another solvent parameter of particular importance is water content and a number of studies have addressed the effect of water thermodynamic activity a_w or concentration c_w on reactions with lipases.[15–19]

Our main aim here was to study the effect of a new type of immobilization medium: aerogels. While silica xerogels are dried by evaporation, aerogels are dried by the supercritical method, with the enzyme already encapsulated in the aerogel. Raising the liquid in the gel to a temperature beyond its supercritical point before drying has the effect of greatly attenuating (in theory suppressing) the capillary stresses that compress both the gel network and the enzyme during drying. This process usually has a minor effect on the specific surface area of the gel, which is not much different in aerogels compared to xerogels. However, supercritical drying has a drastic effect on the specific pore volume, which is much higher in aerogels (90% of a monolith gel volume)[20] than in xerogels (typically 60–70 vol%). This large pore volume has the practical implication that diffusion of substrates and products in aerogels is not very limited and the enzyme molecules can, in the best case scenario, be considered as homogeneously dispersed, as if they were in a solution.

Four types of silica aerogels were synthesized with the enzyme encapsulated inside the gel before supercritical drying, as explained in the Experimental Section. One type of aerogel was made from only tetramethoxysilane (TMOS) as the silicon precursor, and the three other types from TMOS plus an increasing proportion of methyltrimethoxysilane (MTMS). This choice was made because of the importance of the presence of such hydrophobic functionalities, as shown by Reetz et al.[5–8]

The transesterification of vinyl laurate by 1-octanol was chosen as a simple model reaction, not for its commercial interest, but with the aim of easily comparing the activity of the enzyme inside the aerogel and without the gel by means of a simple gas chromatograph. One possible way to characterize the action of the aerogel is to compare its effect on transesterification kinetics to that of solvents with different hydrophobicity. It was also possible to combine such solvents with the aerogel to

[a] *H. El Rassy, Dr. A. Perrard, Prof. A. C. Pierre*
Institut de Recherches sur la Catalyse
UPR-CNRS 5401, 2 Avenue Albert Einstein
69626 Villeurbanne Cedex (France)
Fax: (+33) 4-7244-5399
E-mail: hrassy@catalyse.univ-lyon1.fr

[b] *Prof. A. C. Pierre*
University Claude Bernard-Lyon I
43 Boulevard du 11 Novembre 1918
69622 Villeurbanne Cedex (France)

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examine how the influence of the solvent was displaced by the effect of the aerogel. The solvents used for this aim consisted of one water-miscible (2-methyl-2-butanol; 2M2B) and one immiscible (isooctane) compound, each in water, with a very low water concentration. Two lipases were also tested, one from *Candida rugosa* (termed CRL), the other from *Burkholderia cepacia* (termed BCL), in mixtures of two organic solvents. The kinetics of the free enzymes were studied first. The lipases were then encapsulated in a series of silica aerogels with different hydrophilic characteristics and the catalysis experiments were repeated. The catalytic behavior of the aerogels was analyzed as a function of their hydrophobic methyl group content, and compared with that of mixtures of hydrophobic and hydrophilic organic solvents.

Results

It has previously been verified that reaction kinetics are not limited by the diffusion of substrates and products inside aerogels of high pore volume, similar to those discussed here.[20] Nevertheless, the technique of pre-equilibration of the solid is important. If a hydrophilic aerogel is first equilibrated with aqueous metal salts at high water vapor activity, a high proportion of the pores are filled with liquid water. Consequently, if this gel is then placed in a water-immiscible organic solvent containing water-immiscible substrates, these substrates have great difficulty in accessing the enzyme. Therefore, a low apparent catalytic activity reflects slow transport kinetics, not necessarily a low intrinsic enzyme activity. Pre-equilibration of an initially dry aerogel in the solvent to be used avoids this problem. The first series of results concerns the transesterification kinetics of free lipase powders.

Blank tests

Three blank tests were run to investigate the kinetics of the free lipases. In the first test, an equimolar solution of vinyl laurate and 1-octanol (1 mmol) was added to 2M2B300w (molar water fraction $N_w = 0.154$; 10 mL) without any enzyme. No reaction was observed after eight days. In the second test, a solution similar to the previous one, but without any 1-octanol, was used. Again, no reaction had occurred after 8 days. In the third test, enzymes were added to a solution similar to that examined in the second test (no 1-octanol). In this case, a chemical transformation was observed with 53% conversion after 24 hours to 97% lauric acid and 3% methyl laurate. The concentration of vinyl laurate decreased, while lauric acid, as well as traces of a compound that we identified by GC–MS as methyl laurate, were formed.

Catalytic tests with 2M2B–water solvents

Typical conversion kinetics of the reaction of the substrate vinyl laurate, catalyzed by free CRL and BCL powders in 2M2B–water solvents with different initial water contents, are reported in Figure 1. Overall, it appears that the reaction was much faster with BCL than with CRL. The yield of a product, for instance of octyl laurate, is defined as the percentage of initial substrate

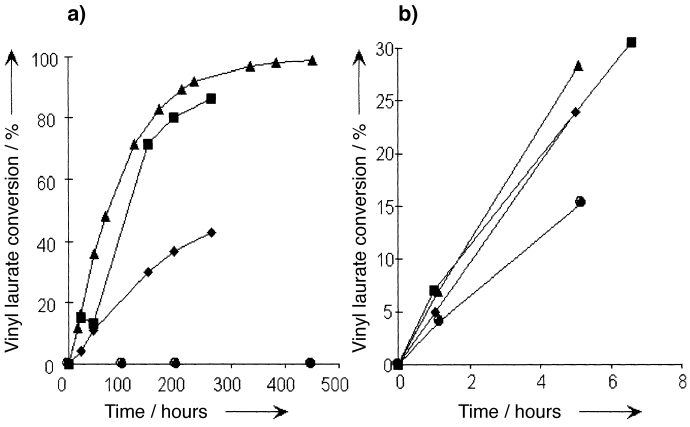


Figure 1. *Example of conversion kinetics of an equimolar solution (1 mmol) of vinyl laurate and 1-octanol catalyzed by free* Candida rugosa *lipase (CRL) and free* Burkholderia cepacia *lipase (BCL) powders. The solvents used were 2M2B solutions (10 mL) with water molar fractions* N_w *of 0.012 (● 2M2B20w solution), 0.057 (◆ 2M2B100w solution), 0.108 (■ 2M2B200w), or 0.154 (▲ 2M2B300w), or water-saturated isooctane (10 mL). a) Conversion of vinyl laurate catalyzed by CRL; b) conversion of vinyl laurate catalyzed by BCL.*

(that is, vinyl laurate) converted into that product. The selectivity for a product is defined as the percentage yield of that product compared to the overall yield of all products formed; thus, the selectivity for octyl laurate = (% octyl laurate formed)/(% octyl laurate formed + % lauric acid formed). This selectivity is represented by the slope of a plot of product yield versus total substrate conversion, as shown by the example in Figure 2, for which 2M2B300w ($N_w = 0.154$) was the solvent and the enzyme

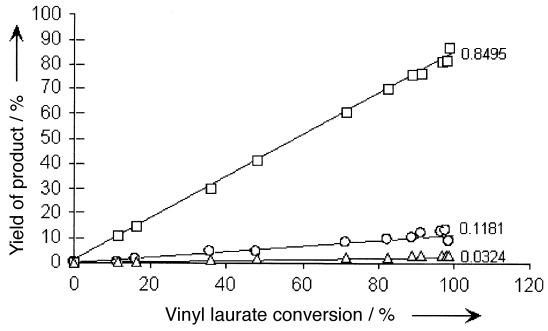


Figure 2. *Example of results demonstrating the linear relationship between the total vinyl laurate conversion into product and the yields of lauric acid (□), octyl laurate (○), and methyl laurate (△). The data were obtained from the reaction with free CRL powder in 2M2B300w* ($N_w = 0.154$; *10 mL). The numbers on each best fit line indicate the slope of the line, which represents the product selectivity.*

was CRL. This figure clearly illustrates the linear relationship found between the yield of each product and the degree of vinyl laurate conversion, with an excellent correlation between these variables up to 100% conversion of the vinyl laurate. Such linear relationships were checked in several cases in the study reported here. This correlation indicates that, for a given enzyme, the selectivity for any product was independent of the extent of vinyl laurate conversion. As a result of these observations and to spare a substantial amount of time, we decided that the kinetics

in most further experiments only needed to be recorded at the beginning of a reaction to provide the selectivities, as well as the initial reaction rates. These kinetics were measured during the first 6 hours of reaction for BCL and during the first 200 hours of the CRL reaction, which is much slower. The global results on the selectivities are reported in Figure 3 as a function of the molar

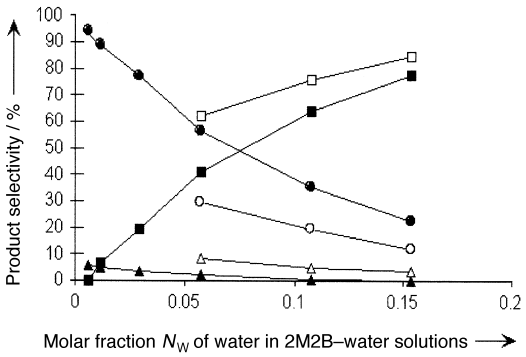


Figure 3. *Selectivities for octyl laurate (CRL: ○, BCL: ●), lauric acid (CRL: □, BCL: ■), and methyl laurate (CRL: ▲, BCL: ▲) in a 1 mmol equimolar transformation of vinyl laurate and 1-octanol catalyzed by free CRL powder and free BCL powder, as a function of the water molar fraction N_w. The solvent was 2M2B – water.*

fraction of water, N_w, in the 2M2B. A similar trend appeared for both enzymes: the selectivity for octyl laurate decreased as the water content increased. Nevertheless, the selectivity for octyl laurate was actually higher with BCL than with CRL. With regard to this figure, it must be mentioned that the vinyl laurate percentage conversion was very small at low water content with CRL (Figure 1 a). The calculated selectivities were therefore meaningless and were not reported in Figure 3 at low N_w values.

The initial conversion rate of the vinyl laurate and formation rate of the octyl laurate measured are shown in Figure 4. In the 2M2B – water mixture, the initial conversion rate of vinyl laurate increased with the water content of the mixture for both enzymes as shown in Figure 4 a, as did the initial formation rate of the octyl laurate for the CRL-catalyzed reaction (Figure 4 b). Since it appeared the enzyme may mainly lose activity in the organic solvent at high water content, it would be interesting to determine an estimate of the enzyme solubility in the various organic-solvent – water mixtures used.

The preceding data on the conversion of vinyl laurate and formation of octyl laurate are consistent with competition between two main reactions. The first reaction is the transesterification of vinyl laurate (LauCOOVin) by 1-octanol (Oct-OH: Scheme 1). The second reaction is the hydrolysis of vinyl laurate (Scheme 2). Both reactions actually produce the enol form of vinyl alcohol (Vin-OH), the acetaldehyde CH_3CHO, which is not able to transform back into the initial substrates of the reactions. Hence these two reactions are not equilibria.

As soon as the octyl laurate and lauric acid had been formed by Reactions 1 and 2, these products underwent the esterification/hydrolysis reaction shown in Scheme 3. Reaction 3 is a combination of the two former processes. That is, Reaction 3 = Reaction 2 − Reaction 1, except that Reaction 3 is an equilibrium because it does not involve the acetaldehyde.

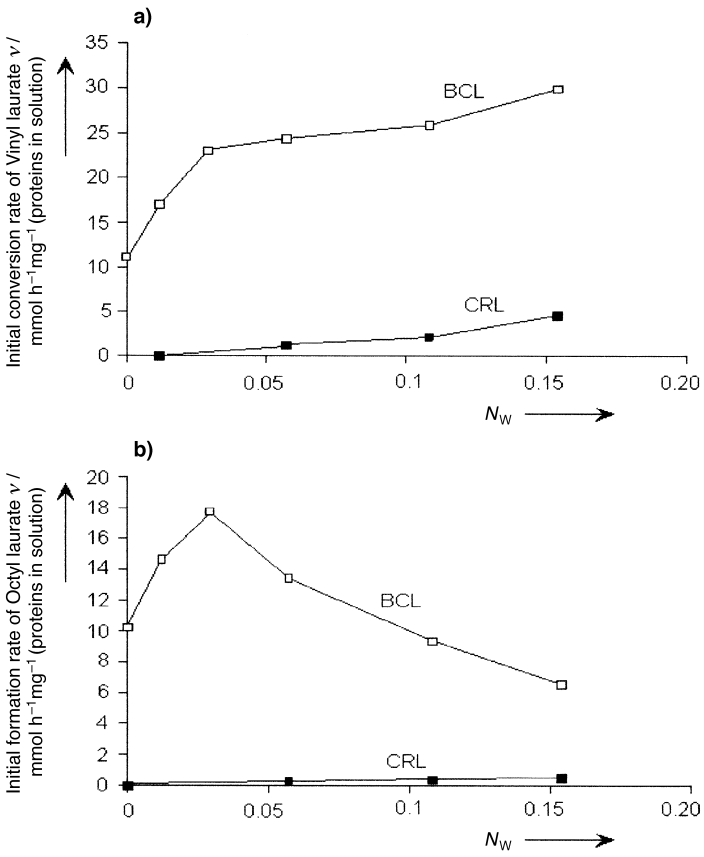
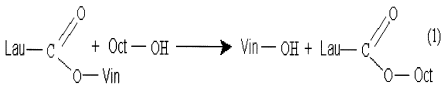
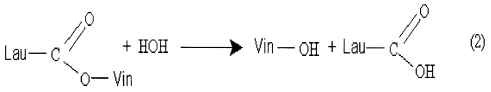


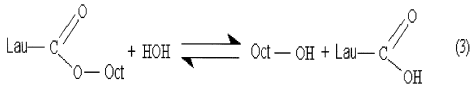
Figure 4. *Initial conversion rate of the vinyl laurate and initial formation rate of the octyl laurate in a 1 mmol equimolar transformation of vinyl laurate and 1-octanol catalyzed by free CRL powder and free BCL powder, as a function of the water molar fraction N_w. The solvent was 2M2B – water.*



Scheme 1. *Reaction 1: The transesterification of vinyl laurate by 1-octanol to give the enol form of vinyl alcohol, the acetaldehyde CH_3CHO.*



Scheme 2. *Reaction 2: The hydrolysis of vinyl laurate, which competes with Reaction 1.*



Scheme 3. *Reaction 3: esterification/hydrolysis of the products formed by Reactions 1 and 2.*

Besides the three main reactions described above, a fourth one occurred that produces residual proportions of a compound identified by GC – MS as methyl laurate. Separate tests actually showed that methanol easily undertakes transesterification

reactions with vinyl laurate. The proportion of methyl laurate formed increased with the proportion of 2M2B in the solvent. Hence, we assume that this fourth reaction is due to transesterification of vinyl laurate with methanol present as an impurity in the 2M2B.

Catalytic tests with ternary solvents

In order to compare the effect of aerogels with differing hydrophobicities on enzyme activity, the activities of the free CRL and BCL powders were determined as described above in ternary solutions of 2M2B, water, and isooctane. Isooctane is a hydrophobic solvent, while 2M2B is hydrophilic. Use of a ternary system makes it possible to modify the hydrophobic – hydrophilic balance in a monophasic liquid solvent. Water was introduced with the 2M2B by using a 2M2B300w solution; the volume percentage of 2M2B300w in the complete solvent volume was varied from 0 – 100 %. In all cases, the total solvent volume was 10 mL.

Both free enzymes showed similar trends with regard to the selectivities as a function of N_w, except that the selectivity for octyl laurate fell slightly faster with N_w increase for CRL than for BCL. Moreover, increased production of the compound previously identified as methyl laurate was observed with increasing N_w value. The initial conversion rates of the vinyl laurate with these free enzymes are compared in Figure 5 and the initial formation rates of the octyl laurate and selectivities for each product are represented in Figure 6. The final extent of conversion of the vinyl laurate was 100 %. These results show that the conversion rate of the vinyl laurate was again much higher with BCL than with CRL (Figure 5 a). However, the most remarkable result concerns the existence of a critical water molar fraction N_w ($\approx$ 0.025), which enhanced the free BCL activity (Figure 5 b). This reproducible enhancement was not due to the combination of isooctane and 2M2B. Indeed, Figure 5 c concerns reactions in dry 2M2B – water – saturated-isooctane (only a trace amount of water present at saturation) and shows that the ratio of 2M2B to isooctane had only a minor effect on the catalytic activity of free BCL. Figure 6 a and b show the initial formation rate of the octyl laurate, which was much higher with BCL than with CRL. Free BCL shows an enhanced octyl laurate formation rate at $N_w \approx 0.025$ (Figure 6 b).

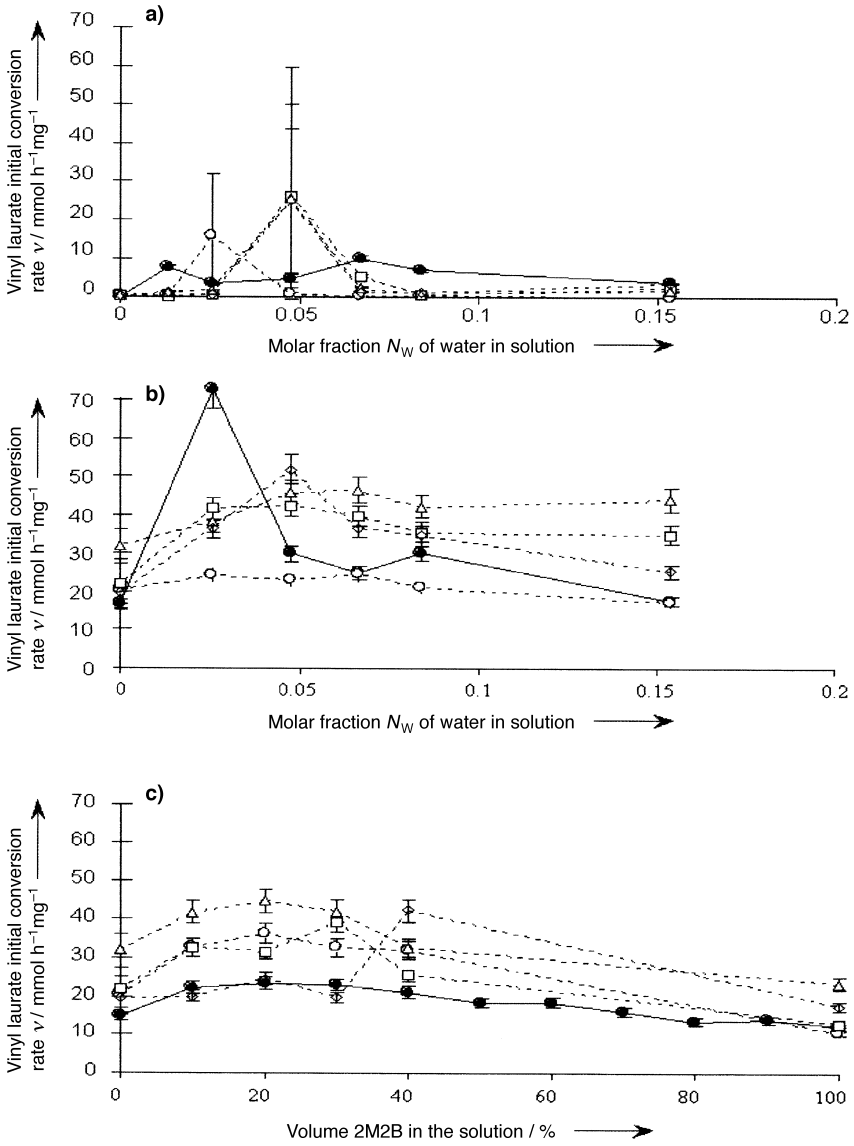


Figure 5. *Initial conversion rate ν of vinyl laurate in a 1 mmol equimolar transformation of vinyl laurate and 1-octanol, for lipases encapsulated in silica gels with different functionalities, as a function of the water molar fraction N_w in 2M2B300w – isooctane solutions: a) with CRL; b) with BCL; c) as a function of the proportion of 2M2B in 2M2B – water saturated isooctane solutions with BCL (●, free lipase; ○, 0 % MTMS; □, 20 % MTMS; △, 40 % MTMS; ◇, 60 % MTMS).*

The results for the same transesterification reaction carried out in the same ternary combinations of solvents as described above but with the encapsulated lipases, are reported in the same figures as the results for the free enzymes (Figure 5 for the initial conversion rate of vinyl laurate, Figure 6 for the initial formation rate of octyl laurate). The encapsulation media were a series of aerogels synthesized with molar ratios of silicon precursors MTMS/(MTMS + TMOS) of: 0 %, 20 %, 40 %, and 60 %.

The presence of the aerogel and its nature only had a moderate influence on the selectivities with either enzyme. In all cases, a rapid decrease in octyl laurate selectivity was observed as the water content in the solvent increased, a trend quite similar to that observed with the free enzymes. Tests were also carried out in a water – saturated-isooctane – 2M2B solvent combination (only a trace amount of water was present in the saturated isooctane). Under these conditions, a slight decrease in octyl laurate selectivity could be seen when a more hydrophilic gel was used. The slight compromise in selectivity was due

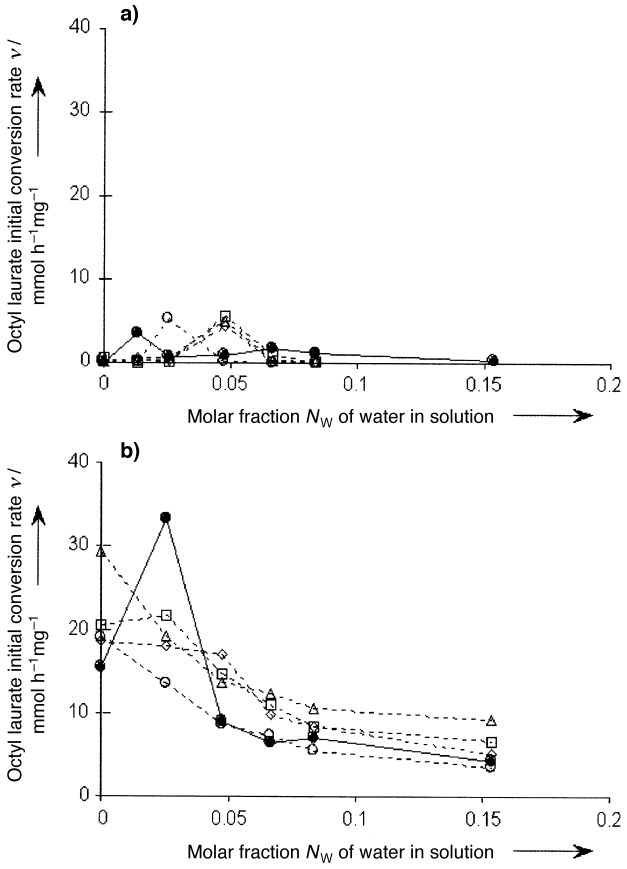


Figure 6. *Initial formation rate ν of the octyl laurate in a 1 mmol equimolar transformation of vinyl laurate and 1-octanol for lipases encapsulated in silica gels with different functionalities, as a function of the water molar fraction* N_w *in 2M2B300w – isooctane solutions for: a) CRL; b) BCL (● free lipase; ○ 0% MTMS; □ 20% MTMS; △ 40% MTMS; ◇ 60% MTMS).*

in part to hydrolysis of vinyl laurate (formation of lauric acid) and in part to an increased formation of methyl laurate in comparison to the amount produced with the free enzyme. The appearance of the latter ester could only be due to an increasing amount of methanol as an impurity, which could originate from the aerogels as these were made from methoxy precursors.

The conversion rate of the vinyl laurate (Figure 5a), as well as the initial octyl laurate formation rate (Figure 6a), with the encapsulated CRL go through a maximum near $N_w \approx 0.025$ for the most hydrophilic-type aerogel (0% MTMS), or $N_w \approx 0.05$ for the other aerogels. However, these maxima were only erratically reproducible on samples with apparently the same drying and preparation history. Hence, a huge uncertainty bar occurred with CRL. This effect could be attributed to inevitable variations in the gel structure of small samples, given the complex steps a gel must go through during preparation. Some gel processing parameter which we have not determined at present is apparently critical for this enzyme, possibly something related to the fact that CRL is about twice as big as BCL.

This problem was not encountered with BCL (Figure 5b). The conversion rate of vinyl laurate (Figure 5b and c) and the formation rate of octyl laurate (Figure 6b) were significantly better with the aerogels than with the free enzyme, either in dry solvents ($N_w = 0$), or at a water molar fraction of $N_w = 0.05$. In these two water content domains, the best aerogel was made from 40% MTMS. The sharp maximum observed with the free enzyme for $N_w \approx 0.025$ disappeared for the aerogels, except for the most hydrophobic aerogel (made from 60% MTMS), which showed an attenuated maximum near $N_w \approx 0.05$ in the vinyl laurate conversion data (Figure 5b).

Discussion

The results reported herein show that aerogels constitute extremely porous media that clearly have an influence on lipase activity. First, as has been observed for other immobilization supports,[21] such media can quite significantly enhance the catalytic activity in dry solvents or in solvents with a higher water molar fraction ($N_w = 0.05$), as shown in particular for the aerogels made from 40% MTMS by their vinyl laurate conversion (Figure 5b and c) and octyl laurate formation (Figure 6b) rates. Second, the balance of hydrophobic and hydrophilic functionalities in the aerogel has an influence as important as that of the proportions of hydrophobic versus hydrophilic liquid organic solvents in which the enzyme is immersed. Third, the aerogel network modifies the influence of a given water thermodynamic activity for a given liquid solvent. Indeed, for a given isooctane – 2M2B – water liquid solvent composition, which always provides the same water thermodynamic activity (even inside the aerogel, as the aerogel was equilibrated with the solvent), the catalytic activity of the enzyme is different depending on whether this enzyme is located inside the aerogel or is free.

With the aim of proposing a mechanism by which aerogels can have such an effect, even at a given water thermodynamic activity, it is interesting to summarize the structure of such aerogels under the conditions used to produce the wet gels before supercritical drying. The structure can be summarized based on the results of studies on sol-gel materials. Silica gel networks are well known to be created by hydrolysis and condensation reactions of the type:

$$Si(OCH_3)_4 + H_2O \Leftrightarrow Si(OCH_3)_3(OH) + CH_3OH$$

$$Si(OCH_3)_3(OH) + Si(OCH_3)_3(OH) \Leftrightarrow (H_3CO)_3SiOSi(OCH_3)_3 + H_2O$$

However, the reactions are much faster with TMOS than with MTMS under the near-neutral hydrolysis conditions used here.[22] The resulting effect can be viewed roughly as presented schematically in Figure 7. First, TMOS forms silica colloidal particles which are, on the one hand, rather dense particles, and on the other hand, loosely interconnected with each other by silica polymers. MTMS then undergoes polymeric condensation with each previously formed colloidal particle, which ends up coated by a crown of branched silica polymers with both hydrophilic Si–OH and hydrophobic $Si–CH_3$ terminations. As the proportion of MTMS increases, the rather dense colloidal silica particles become scarcer and smaller, while the crown of hydrophobic branches becomes thicker and more extensive. Above 40% MTMS (that is, observed in this study with 60% MTMS), an important fraction of the MTMS is not hydrolyzed

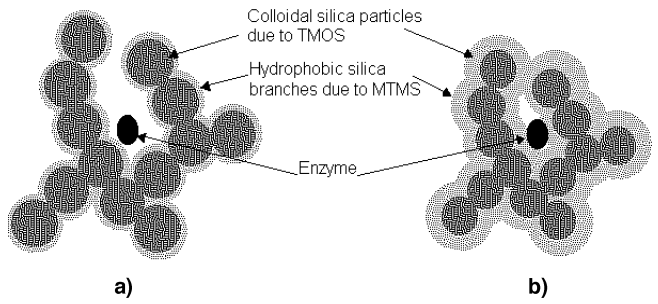


Figure 7. *Illustration of a possible aerogel network texture with enzyme encapsulated in materials made with different proportions of MTMS and TMOS.*

simply because water cannot access some of it. In this case, a xerogel (gel dried by evaporation) appears viscous. In an aerogel, some liquid MTMS is dissolved in acetone before CO_2 supercritical drying and about a third of the silicon from this precursor is lost. The resulting aerogel characteristics are well illustrated by the nitrogen adsorption study summarized in Table 1. No specific

Table 1. *Textural characteristics of the aerogels determined by nitrogen adsorption.*[a]

MTMS [%]	A_{sp} [$m^2 g^{-1}$]	C	$A_{sp,Kel}$ [%]
0	836.8	193	79.6
20	825.6	67	60.6
40	737	19	58.4
60	*387.8*	*−66*	*7.8*

[a] A_{sp} is the specific surface area and C is the constant determined by the BET method (Lowell and Shields, 1991). $A_{sp,Kel}$ is the contribution to the specific surface area made by pores with a radius larger than the Kelvin radius.

pore volume is indicated in this table because it is well established that the largest contribution to the pore volume, which comes from the largest pores, cannot be taken into account by nitrogen adsorption. Hence, such data are generally speaking absolutely meaningless for aerogels.[23] Nevertheless, up to 40% MTMS (inclusive), the specific surface area decreased moderately as the MTMS content used in aerogel synthesis increased. The contribution of mesopores to this surface area decreased somewhat more in proportion to the MTMS content. But the salient figure is the Brunauer Emmett and Teller (BET) constant C, which decreased drastically as MTMS content increased.[24] This constant represents the polarity of the adsorbing surface and the fact that it decreased when the percentage of MTMS increased is to be expected because the proportion of Si–CH_3 versus Si–OH terminal groups increases with MTMS content. Moreover, this polarity essentially disappears at 60% MTMS, resulting in a negative, meaningless C value.

Nevertheless, the negative C value has implications; it indicates that liquid nitrogen would not adsorb onto the pore surface of gels made with 60% MTMS. Liquid nitrogen did not wet such gels. Even water does not wet such a gel as an aerogel sample floats indefinitely on water. In fact, no reliable nitrogen adsorption isotherm could be determined for such samples. Each data point on the adsorption isotherm would have taken a very long time to reach equilibrium adsorption, so that data acquisition was stopped after 3 hours for each point. The values reported in italics in Table 1 correspond to the forced calculations from these nonequilibrium data points. In terms of aerogel texture, this result also indicates that the volume accessible to nitrogen comprised bottle-necked pores with an extremely narrow entrance. In any case, the nitrogen volume adsorbed was very small. On the other hand, the 60% MTMS aerogel could adsorb hydrophobic organic liquids such as isooctane, which explains that enzymes in this aerogel could catalyze a reaction in such solvents.

These analyses suggest that an aerogel network could modify the activity of an enzyme in a given organic solvent for a given water activity in two ways. First, during sol-gel fabrication the enzyme is dispersed in a solution of hydrolysis water. The fact that no turbidity is observable by visual examination after mixing with the silica precursors means that no extensive aggregation of the enzyme occurred. Growing the gel network around the enzyme molecules blocked the enzyme into a well-dispersed state. This good dispersion was maintained after supercritical drying, which avoided any compression of the enzymes by the capillary drying stresses (contrary to drying by evaporation). This good dispersion was also maintained after immersion in an organic solvent, while the free enzyme might otherwise have undergone partial aggregation. Hence, even at a given water activity, the enzyme appears more active than the free enzyme because of these different aggregation states. This explanation is quite consistent with the better activity observed in organic solvents without water (Figure 5c and data point at $N_w = 0$ in Figure 6b) than in solvents with water. Even with 60% MTMS, an activity at least as good as the free enzyme is observed, in spite of the reduced pore volume.

Secondly, in the same way as nitrogen has difficulty entering a very hydrophobic aerogel made with 60% MTMS, other polar reactants or products may have difficulty moving out of or into the gel network, in particular water. Hence, an effect observed with the free enzyme in a liquid medium as a result of the presence of water (such as an apparent optimum water molar fraction N_w) may be expected to be distorted when the enzyme is trapped in the aerogel network. This is consistent with the observations represented in Figure 5a and b and Figure 6a. In this case, the modification of enzyme activity is caused by transport phenomena.

Conclusions

The study reported herein addresses a simple model transesterification reaction catalyzed by lipases (CRL and BCL) encapsulated in silica aerogel dried by the CO_2 supercritical method. Comparison between the effect of aerogels and that of organic solvents, in both cases with varying hydrophobicity, has shown that an aerogel can modify the enzyme activity for a given water activity by two types of mechanisms. First, an aerogel can maintain the enzyme in a dispersed state better than might be possible in the same organic liquid without the aerogel. Secondly, an aerogel can modify the transport of reactants and products of different polarity. The objective now is

to control this mechanism and to apply it to an enzyme and a transformation of commercial value. A possible ultimate aim is that aerogel particles containing the enzyme could be used in the same way as microemulsions, to monitor the performance of enzymes with interfacial activation characteristics.

Materials and Methods

Materials: Lipase from *Candida rugosa* (30 U mg^{-1}) and lipase from *Burkholderia cepacia* (40 U mg^{-1}; previously known as *Pseudomonas cepacia*) were from Amano; vinyl laurate (99%), 1-octanol (~98%), 2-methyl-2-butanol (~98%, 2-methyl-3-butanol ~1%), isooctane (99.5%), tetramethoxysilane (98%), tris(hydroxymethyl)aminomethane (Tris; 99.8%), ammonium sulfate (99.8%) from Fluka; lauric acid (99.8%) from Acros; methyltrimethoxysilane (98%) from Aldrich; hydrochloric acid (37%) from Prolabo; methanol (99.8%) from R. P. Normapur; BCA protein (Reagents A and B) from PIERCE; polyvinyl alcohol (MW 15 000) from Fluka; technical grade acetone, distilled and ultra pure water.

Protein solutions preparation: Enzymatic solutions were prepared in the following ways. BCL free enzyme powder (typically 1.6 g) was dispersed in Tris–HCl (20 mL) buffer solution at pH 7.5, but the powder was only partially soluble. For this reason, the undissolved portion was removed by centrifugation. The supernatant solution, hereafter termed "protein solution", was purified by precipitating the enzyme with ammonium sulfate according to a technique described by Secundo et al.[21] The precipitate was recovered and dissolved in Tris–HCl (10 mL) and the solution was dialyzed for 48 h against distilled water, with a dialysis membrane with a molecular weight cut-off of 10 000. The resulting solution is termed "enzymatic solution" hereafter and was used directly to synthesize aerogels without further preparation steps such as freeze-drying. In the case of CRL, the total amount of free enzyme powder used dissolved in water. Typically, free enzyme powder (about 43 mg) was first dissolved in distilled water (3 mL) to give a solution with a faint yellowish color, termed "protein solution".

The protein concentrations of these solutions were determined by UV/Vis spectrophotometry with calibrated standard proteic solutions, prepared by using Reagents A and B of the BCA protein test. Overall, from an initial mass m of free BCL powder, the total mass of protein recovered was about 0.05m in the protein solutions and around 0.005m in the enzyme solutions. The corresponding numbers for an initial mass m of CRL were approximately 0.05m (which means that most of the soluble free powder was not protein) and 0.0025m, respectively. These numbers were used to compare the results obtained directly with free enzyme powders, with protein solutions and with enzymatic solutions.

All data on gels with encapsulated BCL were collected from BCL enzyme solutions with a concentration of about 0.35 mg enzyme per mL. However, further catalytic tests with BCL protein solutions showed that the catalytic activity was entirely due to the amount of protein corresponding to that of enzyme in the equivalent enzymatic solution. That is to say, for any given initial conversion rate ν per mg "protein" in solution, the equivalent reaction rate per mg "enzyme" in solution was about 10ν. Similarly, with CRL, the equivalent reaction rate per mg of enzyme in solution was about 20ν. In practice, all presented data for CRL were obtained on gels made with CRL protein solutions at a concentration of around 0.75 mg protein per mL.

Enzyme encapsulation: Enzyme encapsulation was carried out in silica aerogels synthesized with the proportions of reactants given in Table 2 in the following way: Methanol and either TMOS and MTMS, or TMOS alone for the hydrophilic gel, were mixed with ammonia solution (0.1 M) in the first pill for 15 minutes. In another pill, the enzymatic solution was mixed with 4% polyvinyl acid (PVA) aqueous solution for 15 minutes. The two solutions were then mixed with each other for approximately 2 hours so that any emulsion eventually disappeared and the whole sample became homogeneous. After mixing, the solution was left to rest for about 24 hours to allow it to form a gel. The gels formed were soaked in acetone for another 24 hours, which allowed water to exchange for acetone inside the gels. This preparatory stage was necessary to allow the further exchange of acetone for liquid CO_2, followed by drying under supercritical conditions (CO_2 critical point temperature and pressure $T_c = 31.4\,^{\circ}C$, $P_c = 7.37$ bar). Aerogels were obtained after drying.

Catalytic tests: The substrates for the catalytic tests comprised vinyl laurate $CH_3(CH_2)_{10}(CO)O(CH)=CH_2$ (1 mmol) and 1-octanol $CH_3(CH_2)_7OH$ (1 mmol). These substrates were dissolved in solvent (10 mL) to which either free enzyme powder (4 ± 0.5 mg) or a gel (typical gel mass ≈ 200 mg) containing water-soluble CRL protein (≈ 0.14 mg) or water-soluble BCL enzyme (0.07 mg) were added, inside a glass bottle (30 mL). The solvents used were either 2M2B containing water at different initial molar fractions, water-saturated isooctane, or a mixture of these two solvents. The glass bottle was placed in an agitated bath from Bioblock operating at 180 rpm and 30 °C. At various time intervals, aliquots (50 μL) were taken and analyzed after dilution in isooctane (750 μL). Analysis was performed on a Shimadzu GC-14B gas chromatograph (GC) equipped with an SGE BP21 capillary column (polyethylene glycol, polar, 12 m long, 0.22 mm inside diameter) and a flame ionization detector (FID). A constant sample volume was injected into the GC with an automatic sample passing system. Absolute calibration of the GC was done by injecting solutions with a known product concentration and readings were found to remain very stable over the experimental timescale. The injector and detector temperatures were 200 °C and 220 °C, respectively. Helium was used as the carrier gas. The temperature program used was: 100 °C for the initial 5 minutes, followed by heating to 190 °C at 10 °C min^{-1}, 5 minutes at 190 °C, and finally cooling to 100 °C.

The results on the effect of water are reported as a function of the water molar fraction N_w ($0 \leq N_w \leq 1$) in the solvents. In solvents miscible with water, as used here, N_w can be easily fixed exper-

Table 2. *Proportions of silicon precursors, solvent, enzyme solution, additive (PVA solution), and gelation catalyst (aqueous ammonia) used for the synthesis of the silica aerogels.*

MTMS/MTMS + TMOS [M]	Methanol [μL]	TMOS [μL]	MTMS [μL]	Aqueous NH_3 (0.1 M) [μL]	Protein[a] or enzyme[b] solution [μL]	PVA solution [μL]
0%	220	410	0	0	192	150
20%	220	328	82	0	192	150
40%	220	246	164	10	182	150
60%	220	164	246	20	172	150

[a] Protein solution of CRL. [b] Enzyme solution of BCL. See the Experimental Section for further explanation.

imentally with accuracy. Since silica aerogels are such porous materials, each of them can be rapidly equilibrated with the solvent simply by immersion in it. For ideal solutions, the thermodynamic activity of water $a_w = N_w$. Although thermodynamic tables for the solutions used here were not available, water was always in dilute proportion ($N_w < 0.154$) so Henry's Law indicates that a_w can be approximated by a linear relationship of the type $a_w = kN_w$, where k is Henry's coefficient. This coefficient could be evaluated independently, together with the exact thermodynamic conversion data, if needed. Reporting data as a function of N_w is thus essentially equivalent to reporting them as a function of a_w, at least for determination of whether particular solution components have an effect on the catalytic activity. However, water is virtually immiscible with isooctane. Hence, when this compound is equilibrated with saturated water vapor (relative humidity 100%), the thermodynamic activity of water $a_w \approx 1$, although the molar fraction N_w is very small. This very limited water content is sufficient to ensure good catalytic activity of the *Burkholderia cepacia* lipase. When 2M2B – water solutions were used as the solvent, the initial water molar fractions in 10 mL 2M2B were $N_w = 0.012$ (20 μL water, solvent termed 2M2B20w), 0.057 (100 μL water, solvent termed 2M2B100w), 0.108 (200 μL water, solvent termed 2M2B200w), or 0.154 (300 μL water, solvent termed 2M2B300w). The mixed isooctane – 2M2B – water solutions used as solvents were obtained by mixing isooctane and 2M2B300w in various proportions, to give a total volume of 10 mL. In tests with the free enzymes and a mixture of solvents, it was not possible to determine the concentration of soluble enzyme because it was not possible to make standard protein solutions in these solvents. However, the stage of solution preparation at which the enzyme powder is dissolved is very important. If the powder is added to an isooctane – 2M2Bw solution, it forms a fine suspension, which separates out to give a fine powder after shaking is stopped. On the other hand, if the enzyme powder is added to one of the solvents, it forms a fine suspension and when the other solvent is added the enzyme agglomerates and forms coarse agglomerates at the bottom of the reactor. In all our tests we used the first method, in which the enzyme powder forms a fine suspension upon shaking.

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