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A novel support for enzyme adsorption: properties and applications of aerogels in low water media

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Abstract

Aerogels, because of their porosity, have a great ability to adsorb water, and this characteristic was exploited to extend the applicability of aerogels for the adsorption and entrapment of hydrolases to be used in organic media. The applicability of aerogels as solid supports for immobilisation of the enzymes PGA, thermolysin and α-chymotrypsin was assayed. By controlling the distribution of water between the catalyst, the support and the reaction medium, aerogel preserves the catalytic activity of the enzymes and prevents hydrolytic reactions.

Aerogels are advanced materials that consist normally of more than 96% air, while the remaining 4% is a matrix of silicon dioxide. Aerogel, consequently, is one of the lightest solids ever conceived and it has unique properties such as high porosity, large surface area, low density and low thermal conductivity. Moreover, silica aerogels present an extremely large number of accessible hydroxyl groups, and consequently show strong hydrogen-bonding effects. All these properties make silica aerogels effective materials, in for instance the production of microfiltration membranes or adsorbents.[1]

Studies on the adsorption of water on porous materials indicate that motion of water molecules in silica based matrixes strongly depends on the pore dimensions.[2–5] There are two types of water in the pores.[4] One type of water behaves like bulk water, probably present in the central region of the pores, and the other type is water perturbed strongly by the silica surface. Consequently, pores of different sizes have different relative amounts of bound and free water.[3–5]

Recently we demonstrated that penicillin G amidase (PGA) and proteases such as thermolysin and α-chymotrypsin are active in low-water media, at controlled water activity, when adsorbed

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on hydrated Celite R-640,[6] a porous silica matrix which can adsorb large quantities of water (>100% of its weight).[7]

Aerogels, because of their porosity, have a great ability to adsorb water. The aim of this study is to verify the applicability of aerogels, for the adsorption and entrapment of hydrolases to be used in organic media at controlled water activity.

The applicability of aerogel as a solid support for immobilisation of the enzymes PGA, thermolysin and α-chymotrypsin was assayed.

Preparation and characterisation of the aerogel

The aerogel was prepared in three main steps:[†]

1. Alcogel synthesis: a base catalysed (NH_4OH, NH_4F) polymerisation of the tetraethyl orthosilicate (TEOS) with water in ethanol.

$$(Si(OCH_2CH_3)_{4(liq.)}+2H_2O \rightarrow SiO_{2(solid)}+4HOCH_2CH_{3(liq.)})$$

2. Aging: the gel is covered with an alcohol/water (3/1) mixture for at least 72 hours to allow a strengthening of the silica network.
3. Drying, aerogel formation and solvent removal under vacuum.

The aerogel prepared following this procedure was characterised. The surface area of aerogel (672 m^2/g) and the micropore surface (11 m^2/g) were calculated using the B.E.T. technique (Brunauer–Emmet–Teller). The data obtained indicate a micropore percentage of 1.64% (diameter<30 Å). The shape and dimension of the fragments of aerogel are not homogeneous (300 μm–3 mm).

Aerogel (200 mg) in 1 mL of dry toluene leads to a water activity of 0.4 due to the percentage of water that cannot be removed during the drying step. Upon addition of water to the solvent, the silica matrix presents a great ability to adsorb water, maintaining the a_w between 0.75 and 0.87, namely from 20 to 200 μL in 1 mL of toluene.

PGA adsorbed onto silica aerogels

The aerogel was previously hydrated with 50 μL of buffer or ultrapure water in toluene.[‡] After 24 h, the enzyme, solubilised in either 100 μL of ultrapure water or aqueous phosphate buffer (pH 8), at two different concentrations (0.1 and 1 M), was adsorbed onto the siliceous matrix by adding the enzymatic solution to the toluene containing the aerogel, according to a method previously described.[6] After 24 h of equilibration the observed values of water activity were in agreement with the necessary hydration to maintain PGA active in organic solvent: i.e. $a_w = 0.83–0.87$.[8]

The synthetic activity of the enzymatic preparation was studied in the acylation of L-TyrOEt (**1**) with methyl phenylacetate (**2**) in toluene. All the preparations obtained have a good activity in toluene and all reactions reached complete conversion in 5–10 hours and no hydrolytic reaction was observed. The enzymatic preparation adsorbed using a 0.1 M phosphate buffer, to solubilise the enzyme, gave an initial rate of 2.09 μmol h^{-1} U^{-1}. It is noteworthy that PGA adsorbed on Celite R-640 following the same procedure[6] leads to an initial rate of 0.98 μmol h^{-1}

[†] Experimental conditions: TEOS 30 mL, ultrapure water 21 mL, ethanol 60 mL, NH_4OH 0.37 mL, NH_4F 0.36 mL.
[‡] Experimental conditions: reaction volume = 1 mL of dry toluene, $T = 30°C$; [**1**] = 80 μmoles, [**2**] = 100 μmoles, 6.2 mg/mL of lyophilised PGA (activity = 9.6 U/mg of protein).

U^{-1}, in the same range of water activity. This difference is most probably due to the larger surface area of aerogel (672 m^2/g), if compared to Celite R-640 (65 m^2/g). Adsorption on aerogels translates into an improvement of the dispersion of the enzyme and of the enzyme-substrate interaction.

Proteases adsorbed onto silica aerogels

The activity of thermolysin and α-chymotrypsin, adsorbed onto the same matrix, in toluene, was studied. The activity of both enzymes in organic media is deeply affected by the degree of hydration.[6] A major limit to the achievement of high yields is due to the production of water during these reactions.[9,10] In this connection, the aerogel has been used as an alternative method for removing the excess water produced during the reactions because of its high water-binding capacity. The hydrolases were adsorbed onto the aerogel using the same procedure of adsorption as described for PGA. The reaction between the acyl donor Z–L-Phe–CO_2H and some nucleophiles was studied (Table 1).

Table 1[a]

Enzyme[b]	[Z–L-Phe–CO_2H], μmoles	Nucleophile	a_w	Conversion (144 h)[c]
Thermolysin	100	L-Phe–OEt[d]	0.80	84
Thermolysin	100	L-Tyr–OEt[d]	0.83	45
Thermolysin	100	L-Leu–NH_2[d]	0.83	77
α-Chymotrypsin	80	MeOH[e]	0.82	54
α-Chymotrypsin	100	L-Tyr–OEt[d]	0.83	30

[a] Experimental conditions: reaction volume = 1 mL of dry toluene, $T = 30°C$.
[b] 5 mg/mL of enzyme (activity of thermolysin = 42 U/mg, activity of α-chymotrypsin = 68.6 U/mg).
[c] Conversions were evaluated by titration of the Z–L-Phe–CO_2H with 0.01 M NaOH.
[d] [Nucleophile] = 80 μmoles.
[e] [MeOH] = 100 μmoles.

In contrast with the reactions using enzymes adsorbed onto Celite R-640, in all the reactions described no complete conversion was achieved after 144 hours of reaction time.[6] This can be probably ascribed to diffusion limits in the reaction system between the enzyme and the substrates. It is remarkable that the a_w of the systems remained at a constant value ($a_w = 0.80$–0.83) during the course of the reactions, making the silica aerogels suitable as supports in synthetic reactions liberating water.

PGA entrapped in aerogel

In order to exploit the innovative potential of aerogel as a support for biocatalysts, the entrapment of PGA on aerogel was also studied.[§]

To entrap PGA in the aerogel, 29.4 mg of the enzyme was solubilised in ultrapure water (21 mL) and added during alcogel synthesis step. The obtained enzymatic preparation, assayed in water, showed a ten time decrease of enzymatic activity, when compared to the native enzyme. The same enzymatic preparation gave no reaction at all in toluene, even working at $a_w = 0.83$–

[§] Experimental conditions: reaction volume = 1 mL of dry toluene, $T = 30°C$, [**1**] = 80 μmoles, [**2**] = 100 μmoles, 200 mg of PGA/aerogel.

0.87. The a_w of the system was adjusted by adding 100 μL of water and phosphate buffer at different pH (pH 7, pH 8 pH 9). The loss of activity of the PGA entrapped within aerogel is probably due to diffusion barriers, since the entrapped enzyme is not easily accessible by the organic solvent and substrates.

Conclusions

Silica aerogel is a solid matrix suitable for the adsorption of enzymes applicable in organic media, whereas the entrapment of the enzyme within the aerogel causes loss of catalytic activity, both in toluene and water. Silica aerogels are hygroscopic supports and their adsorption capacity can be exploited in synthetic reactions, liberating water to prevent the competition of hydrolytic reactions (esterification of amino acids and peptide synthesis). The degree of fragmentation of aerogel must be optimised, in order to obtain aerogel samples with reproducible properties (porosity, shape, adsorption capacity). These promising preliminary results induce to pursue the study of the properties of aerogels. In particular, the porosity of these supports and their great capacity of adsorbing water make this type of porous support potentially applicable in biotransformations in non-aqueous media at controlled water activity.

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