Thylakoids and chloroplasts entrapped into porous silica gel

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INTRODUCTION

Due to its high degree of sophistication, selectivity, functionality, complexity and architecture, nature is really a school for materials science, chemistry, biology, physics and engineering. One of its best examples is the living cells. These ones can be considered as veritable complex molecular engines spatially enclosed. This highly organized system is self-maintained and self-generated internally by metabolic processes controlled by the flow of genetic information. In addition, cells integrate the environmental factors by signal transduction pathways and converge to regulate cell phenotype (Mann S., 2008). Nonetheless, living cells, and more generally biological species, isolated from their native environment are unfortunately fragile or unsuitable to develop systems which perform a particular biological process. Since the *de novo* construction of resistant artificial living cells, oriented to design defined applications, is hardly conceivable for the moment, materials scientists have the smart idea to combine abiotic materials with different living species. This symbiotic combination for both protection and control of biological species has appeared as a key point to develop new kinds of "living" biotechnologies presenting the desired properties (e.g. biosensors, bioreactor and artificial organs).

Facing the uprising problems of depleting fossil fuels and global warming, the development of new "green" technologies becomes a global concern. Many research teams have focused their effort on the conception of sophisticated devices able to convert sunlight into eco-friendly fuel (Armaroli N., 2007). However, the design of a cheap machinery, which is more efficient or, at least, mimic the natural photosystem protein complex found in plants, remains a fundamental challenge (Sanderson K., 2008). An alternative is to develop a hybrid material which combine the integrated complex chemical systems found in biological materials with an abiotic material. Previous studies have predominantly focused on the immobilization of photosyntethic pigments, proteins (Chen Z., 1995) and complexes (Oda I., 2006) within silica matrixes with the view to reproduce the photosynthetic apparatus with the same level of interactions found in living plants. More recently, algae (Fiedler D., 2007), diatoms (Gautier C., 2006) and cyanobacteria (Rooke J., 2008) encapsulated within porous silica gel have been reported to retain their activity, showing that porous silica is also adpated for the encapsulation of more voluminous membranous systems.

The present work summarizes the recent advances to design a stable photobioreactor, by entrapping into a three-dimensional silica network fragile thylakoids; the parts of chloroplasts located in plant cells that harnesses solar energy and are able to convert water into oxygen (Meunier C., 2009). Compared to other biological systems such as bacteria that can resist or adpat to their environments (e.g. presence of alcohols, salts, pH) up to some extend, thylakoids are very sensitive to their surrounding. The aim is thus to protect the thylakoids *via* a protecting silica matrix. Using only the key compartment (thylakoids or chloroplasts) of whole plants, respiration, performed by mitochondria, can be avoided. Therefore, this pure photosynthetic material could have a better efficiency in CO_2 conversion and oxygen production than whole plants.

MATERIALS AND METHOD

Isolation of thylakoids and chloroplasts. All steps dedicated to the isolation of thylakoids and chloroplasts were performed at 2°C in order to preserved the photosynthetic activity. Spinach plants (*Spinacea oleracea*) were first kept in the dark for 24 hours prior to the isolation process. A thylakoid suspension was obtained from a chloroplast extract by a two step process (Meunier C, 2009). The biological buffer used had the following composition: 330 mM sorbitol, 50 mM HEPES-KOH pH 7.8, 2 mM EDTA, 1 mM $MgCl_2$, and 1 mM $MnCl_2$.

Syntheses of photosynthetic hybrid materials. Different sol-gel routes were investigated in order to immobilize thylakoids and chloroplasts in porous silica. Particularly, a biocompatible synthesis pathway has been designed to allow the formation of a robust hybrid silica gel without releasing any by-product during its construction. First, a metastable silica sol was prepared at 0°C by mixing sodium silicate solution (1.5 M) with an acid resin previously rinsed with acid water (Amberlite® IR120). The resulting clear solution was separated from the resin by filtration. Silica nanoparticles Aerosil®) were then added in order to increase the silica concentration. After homogenization, the pH and osmotic pressure were adjusted. Then, the biological suspension (containing either thylakoids or chloroplasts) was added within 35 min, before gelation occurred.

Characterization. Information on the porosity, morphology and texture of hybrid gels was obtained by scanning electron microscopy (SEM), transmission electron microscopy (TEM) and nitrogen adsorption-desorption experiments. The structural and functional preservation of thylakoids and chloroplasts were studied by UV-Visible spectroscopy, confocal microscopy and oximetry (Clark cell vessel). Details about characterization techniques are described in Meunier C., 2009.

RESULTS AND DISCUSSION

Thylakoids as well as whole chloroplasts are not stable in an isolated state. Therefore, one way to exploit their photosynthetic properties is to develop hybrid systems that incorporate biological species and confer protection while maintaining the biological functions. In regards to the immobilization of photosynthetic species, porous silica based system seems to be an ideal host. Indeed, it is quite inexpensive to synthesize, chemically inert, optically transparent, resistant to microbial attack, mechanically strong and thermally stable. Morover, silica is biocompatible as evident from its existance in nature, in the construction of frustules, the exterior cell wall of diatoms. This work demonstrates the first attempts to stabilize and control the environment of fragile thylakoids and chloroplasts by encapsulating them within a silica matrix.

Activity of immobilized thylakoids. Fig. 1 shows the spectra from UV-Visible experiments on hybrid gels aged during 4 days. For the sake of comparison, free thylakoids adsorption curve (FTH) has been collected just after their isolation from plant leaves. In both cases, there are two groups of overlapping bands attributed to the photosynthetic pigments (chlorophylls and carotenoids). Compared to the free suspension, any observable shift of bands from hybrid gel (HG) has been observed. These results indicate that silica does not seem to affect the photophysical properties of the photosynthetic apparatus. Fig. 2 shows the variation in the enzymatic activity of hybrid gel and free thylakoids in a suspension. The activity of free thylakoids declined very rapidly and became undetectable after 3 days. To the sharp contrast, encapsulation in silica gel can significantly extend the enzymatic activity. Even though approximately 35% of the initial activity is lost due to osmotic and shear stresses encountered

during the silica polymerization step, oxygen production can be detected over the course of 40 days at 10°C.

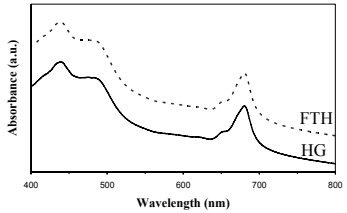

Figure 1: UV-visible spectra of entrapped thylakoids and free thylakoids suspension (FTH)

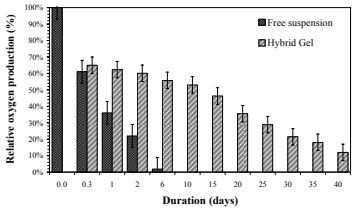

Figure 2: Relative oxygen production by entrapped thylakoids.

Effects of the silica matrix. One important feature concerning the preservation of physiological function of thylakoids is linked to the textural properties of the synthesized matrices for two reasons. Firstly, porosity is essential in order to allow light and matter to permeate through the silica network and then be delivered to the biological systems. Secondly, the silica framework itself could act as a scaffold in some conditions, maintaining the structural organisation of thylakoids. However with aging time, this scaffold could also restrict the environment of thylakoids, placing them under increased stress. Fine control of silica network properties is thus critical to the successful encapsulation of thylakoids.

The choice of silica precursors as well as their concentration can control the textural properties of matrixes. Indeed, the average pore size can be tuned from 1 µm to 150 nm. In all cases, diffusion experiments shown that the accesibility of entrapped thylakoids is large enough to ensure the diffusion of nutrients and the bio-production of oxygen.

Another important parameter that has to be considered in the encapsulation process is the gel synerisis rate which provokes detrimental contraction and shearing strain in the thylakoids. As shown Fig. 3, the gel contraction rate can decrease by the introduction of silica nanoparticles. By introducing silica nanoparticles, the sol already contains a high proportion of quaternary SiO_4 unit, as proved by ^{29}Si MAS NMR. The condensation number of silanol bonds is thus limited after the addition of thylakoids. Nevertheless, the confinement created by the silica cage has to be tight enough to maintain functionally complex membranes in an active form. In an effort to confirm this model, different additives were added during the gel formation. For instance, the introduction of glycerol, a well-known cryoprotectant, seems to impair the structural support of the matrix.

Integrating these parameters, a biocompatible hybrid silica gel has been designed to encapsulate whole chloroplasts. Confocal microscopy was used to obtain structural information of chloroplasts within wet gels. The fluorescent signal clearly shows the beneficial effect of the silica matrix. *Grana* (stacked thylakoids) are well preserved after the encapsulation of chloroplasts (Fig. 4). These organelles are better maintained and remain isolated within the matrix over time (e and f of Fig. 4) while those ones in a suspension show degradation and aggregation (b and c of Fig. 4). The porous silica acts as an artificial membrane supporting the biological structures. Close interactions between thylakoids and silica scaffold are thus essential to preserve their enzymatic activity.

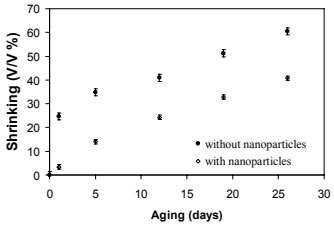

Figure 3: Kinetics of gel shrinking of hybrid gels at 10°C

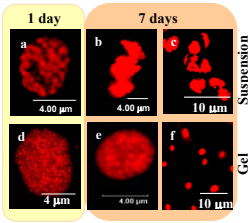

Figure 4: Kinetics of gel shrinking of hybrid gels at 10°C

CONCLUSIONS

The encapsulation of fragile membrane systems is a real challenge. This work demonstrates, using an aqueous sol-gel route, that silica gels can provide an adapted environment to stabilize thylakoids and chloroplasts. The photosynthetic activity of thylakoids, immobilized into a porous three-dimensional silica matrix, can well be maintained over 40 days. Additionally, the structural organization of chloroplasts can not only be preserved after the encapsulation but well maintained over time. It has been shown that the bioactivity of these biological membranes depends on some key factors such as the kinetic of silica polymerization, the chemical environment and the silica density. The present hybrid gels are good examples that may be used in the design of photosynthetic fuel cells and be extended to biosensing applications to detect herbicides and various environmental pollutants.

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