Communication

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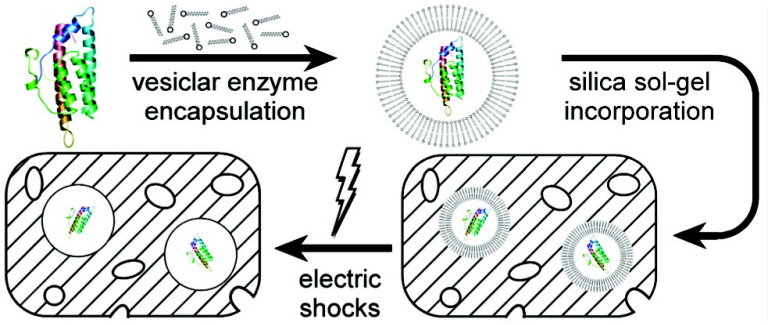

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Liposomes as Protective Capsules for Active Silica Sol−Gel Biocomposite Synthesis

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Silica sol−gel biocomposites are promising materials for the development of biosensors and for biocatalysis. As demonstrated by the original work of Dickey and other pioneers, silica sol−gel has consistently been an excellent medium for the immobilization of active enzymes.[1] Numerous encapsulation strategies have been developed over the years to maximize the activity of an immobilized enzyme. The most common strategies to afford more biocompatible environments include (i) the use of ormosil, (ii) the co-encapsulation of sugar as well as polymeric additives, and (iii) the use of sodium silicate as a precursor.[2] It has also been demonstrated recently that silicatein biomimetics can be used to eliminate harsh chemical reaction conditions, and the successful encapsulation of fragile firefly luciferase was reported.[3] The diversity of the above encapsulation strategies suggests that silica sol−gel bioencapsulation is enzyme specific, presumably due to protein−silica interactions during and after the sol−gel formation process. Any modification to specific protein−silica interactions will have a profound impact on the activity of a resultant biocomposite material. In this communication, we report a bioencapsulation protocol that eliminates specific protein−silica interactions during the sol−gel formation process, producing silica sol−gel biocomposites that are more active than those prepared by trapping enzymes directly in silica hydrogels. Importantly, since the enzyme being encapsulated is completely shielded from the sol−gel reaction, this protocol is expected to be readily adapted to the encapsulation of a wide range of enzymes.

As shown in Scheme 1, the process begins with an enzyme being trapped inside a liposome, which serves as a protective coating to keep the enzyme from reactive silane reagents. Isolation of enzyme from the sol−gel formation process should prevent OH-bearing amino acid residues, such as serine, threonine, and tyrosine, from reacting with silanol and becoming covalently bound. Moreover, any molecular templating effect due to direct contact between enzyme and silica sol while the sol−gel is formed should be effectively eliminated as well. After solidification, the resultant silica matrix is shocked by strong electrical pulses to break apart the liposomes and release the entrapped enzyme for subsequent applications.

Figure 1 shows the emission spectrum of green fluorescent protein (GFP) before and after it was subjected to 30 electric shocks. The data indicate that GFP is unaffected by the shocks. Also shown in the figure are the steady-state fluorescence anisotropy values of GFP measured from hydrogel monoliths with different sol:buffer volume ratios. The anisotropy values suggest that, when directly trapped, GFP has less mobility in a sample that contains less water, presumably due to smaller silica pore sizes. However, when GFP was trapped in liposomes prior to encapsulation, its fluorescence anisotropy value decreased by almost a factor of 2 in both samples, indicating that GFP has a higher mobility when encapsulated by the new procedure. More importantly, the fluorescence anisotropy values of the protected GFP appear to be independent of the sol:buffer volume ratio, even though the internal silica frameworks of the 1:7 and 1:20 samples are very different. This implies that the local environment of GFP in both samples was very similar when the protein was still inside the liposomes. The fluorescence anisotropy values in both samples did not change significantly after the electric shocks. Thus, either the newly released GFP was able to maintain its mobility inside the nascent silica pores or the shocks were too weak to destroy the liposomes, leaving the GFP unperturbed.

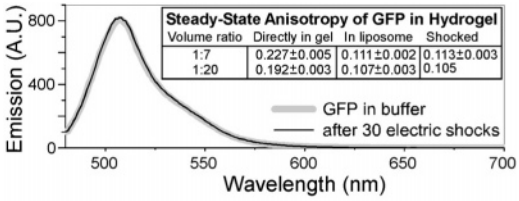
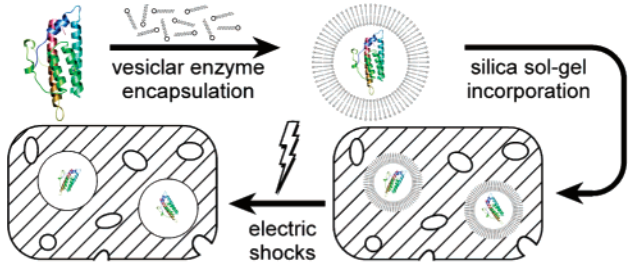


Figure 1. Fluorescence spectrum of GFP in a 10 mM TE buffer, pH 8.0, before and after the application of a 1.5 kV, 20 ms electric shock for 30 times. The table compiles the steady-state fluorescence anisotropy values measured from GFP directly trapped in hydrogels, GFP trapped in liposomes that were subsequently incorporated in hydrogels before and after the application of multiple electric shocks. All measurements were made with a 488 nm Ar^+ laser excitation.

Scheme 1



To determine whether an enzyme can be successfully released from the liposome, horseradish peroxidase (HRP) was encapsulated and its chemiluminescence intensity was measured before and after application of the electric shocks. HRP is a two-substrate enzyme. In the presence of H_2O_2, HRP will oxidize luminol to generate chemiluminescence. It has been reported that HRP can withstand the silica sol−gel process and remains active upon encapsulation.[4] In the present studies, a small decrease in activity in the 1:7 and 1:20 hydrogel samples was observed when HRP was directly trapped. Since the HRP chemiluminescence spectra from our samples resemble those of the enzyme in aqueous buffers, the catalytic cycle of encapsulated HRP is not significantly altered. In addition, although HRP was present throughout the hydrogel, chemiluminescence was limited to the gel surface because of poor substrate diffusion. To produce a stronger signal and to minimize the diffusion problem, HRP-doped liposomes were encapsulated with luminol and H_2O_2 such that both substrates were available

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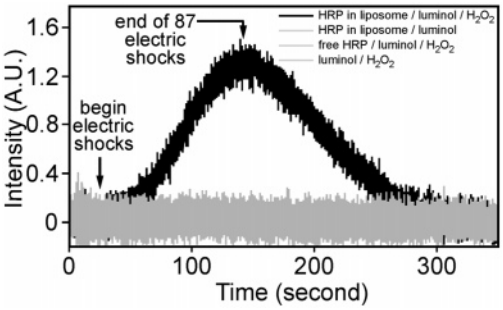

Figure 2. Chemiluminescence intensity time courses monitored at 450 nm from different silica hydrogel samples that were subjected to multiple 1.5 kV, 20 ms electric shocks. Since all control samples produced no signal, their intensity time courses are all represented by the same color.

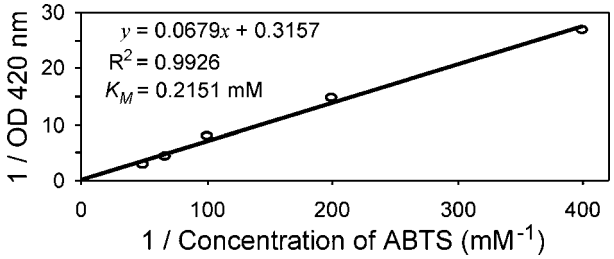

Figure 3. Lineweaver−Burk plot of HRP prepared by the new bioencapsulation protocol. Activity of HRP was assayed after 87 1.5 kV, 20 ms electric shocks were applied. Since HRP is a two-substrate enzyme, the K_M reported above was measured against 1 mM of H_2O_2.

throughout the hydrogel. While preparing the sample, an interesting phenomenon was observed. When silica sol was added to an aqueous buffer that contained luminol, H_2O_2, HRP-doped liposomes, and some residual HRP, chemiluminescence from the residual HRP was rapidly quenched, indicating that unprotected HRP was effectively inactivated. Inactivation of HRP by non-native substrates has been reported.[5] Unlike previous reports, however, this new HRP inactivation process only occurs when the normal HRP catalytic cycle is affected by the hydrogel formation process. We exploited this phenomenon to eliminate background chemiluminescence from residual HRP in hydrogels and examined the chemiluminescence response of liposome-trapped HRP.

Figure 2 compares the time courses of chemiluminescence intensity from different hydrogels while electric shocks were being applied. Only the hydrogel that contained HRP-doped liposomes, luminol, and H_2O_2 produced chemiluminescence. No activity could be detected without H_2O_2, HRP-doped liposomes, or when HRP was not protected. The presence of chemiluminescence is a clear indication that shocking the liposomes brought the initially separated HRP and its substrates together. It is reasonable that chemiluminescence occurred when HRP was released from the liposomes that were destroyed by the electric shocks. Alternatively, the shocks might have caused dielectric breakdown and induced nanopore formation on liposome surfaces, allowing substrates to diffuse inside and react with HRP. High voltage electric pulses on the order of microseconds have been shown to cause electroporation of liposomes.[6] We believe that the much longer millisecond pulses that we repeatedly employed should cause any nanopore to expand irreversibly and eventually to destroy the liposome. This explains why the chemiluminescence in Figure 2 continues for more than a minute after the last shock was applied.

We also examined the kinetics of HRP as a function of the concentration of azinobis(3-ethylbenzothiazoline-6-sulfonate) (ABTS). The Lineweaver−Burk plot in Figure 3 shows that HRP encapsulated by the new protocol exhibits Michaelis−Menten kinetics,

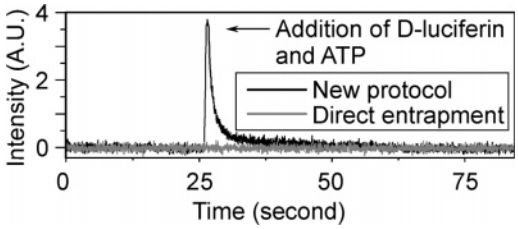

Figure 4. Bioluminescence intensity time courses monitored at 560 nm from unprotected and liposome-protected LUC upon the addition of D-luciferin and ATP. Both hydrogel samples were subjected to multiple 1.5 kV, 20 ms electric shocks before the substrates were added.

similar to the behavior of HRP directly encapsulated in silica hydrogels reported elsewhere.[2c]

Next, we extended our investigation to firefly luciferase (LUC) to test whether the new protocol works with more fragile enzymes. Firefly luciferase is also a two-substrate enzyme, which consumes adenosine triphosphate (ATP) and oxidizes D-luciferin to give off a greenish−yellow bioluminescence. The enzyme is known to denature easily upon direct entrapment into silica alcogels. In Figure 4, we compare the bioluminescence time courses for unprotected and liposome-protected LUC. It is evident from the figure that unprotected LUC is inactive, while the hydrogel prepared from liposome-protected LUC generated a clearly discernible bioluminescence signal.

In summary, we have developed a new encapsulation protocol that is potentially applicable to the entrapment of a wide range of enzymes. Importantly, using liposomes as templates, this protocol allows the immobilization of an enzyme inside silica pores of controlled dimensions. We anticipate that this protocol will lead to investigations that can provide new insights into protein−silica interactions in silica hydrogels.

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Supporting Information Available: Information regarding sample preparation and assay conditions for HRP and LUC. This material is available free of charge via the Internet at http://pubs.acs.org.

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