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Spectrochemical Behavior of Enzymes Encapsulated Within Xerogel

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Abstract

There are many methods that have been used in the encapsulation or immobilization of enzymes. One of them is the sol-gel process, which can be used to produce porous silica materials even at ambient temperature. In this study, several enzymes, namely catalase, lipase, laccase, and glucose oxidase were encapsulated by sol-gel process using tetramethyl orthosilicate (TMOS) as alkoxide. The behavior of the enzymes encapsulated within the gel matrix, or xerogel, was monitored by fluorescence to determine the spectrochemical behavior of the enzymes. The results were compared with the enzymes in solution form. There are minor differences between the spectra in different matrices (xerogel and solution), which can only means that there are conformational changes in enzymes when they were immobilized within the gel matrix.

Keywords: Enzymes; Sol-gel; Encapsulation; Xerogel

Introduction

The sol-gel process is used for the fabrication of glass and ceramic materials, wherein the solution (sol) evolves gradually towards the formation of a gel like network containing both a liquid and solid phase [1]. Sol-gel techniques have become very popular recently due to their high chemical homogeneity, low processing temperatures, and the possibility of controlling the size and morphology of particles. The sol-gel derived materials provide excellent matrices for a variety of organic and inorganic compounds. The advantages of sol-gel technology are used in the fields of biomedical sensors, laser materials, and for sustained drug delivery applications. Hench and West [1] showed that through the use of the sol-gel technique, it was possible to fabricate highly pure and homogeneous materials with controlled pore structure.

The sol-gel technique has been used for fabrication of sensors, catalyst supports, optical elements, coatings and special polymers. Among the materials being encapsulated are biomolecules like proteins, enzymes and catalytic antibodies. These biomolecules can be entrapped in robust silica glasses under mild conditions allowing the retention of their chemical activity [2]. There have been reported studies [3-6] on the ability of proteins and enzymes such as bovine serum albumin (BSA), myoglobin, hemoglobin, copper zinc dismutase and glucose oxidase to undergo the necessary conformational changes for the binding and release of substrate.

In this present work, enzymes such as catalase, lipase, laccase, and glucose oxidase were encapsulated within sol-gel matrix. The main objective of this study is to observe the spectrochemical properties particularly the fluorescence, of the four enzymes when encapsulated within xerogel matrix. The emission profile of the enzymes encapsulated in the xerogel was compared to that of the enzymes in solution form. The effect of aging with respect to the behavior of the enzyme within the gel was also determined.

Experimental Procedure

Tetramethyl orthosilicate (TMOS) (Aldrich), was used without further purification. Lipase from porcine pancreas, catalase from bovine liver, laccase and glucose oxidase from Aspergillus oryzae were purchased from Sigma Aldrich.

The sol solution was prepared by mixing 7.00 mL tetramethyl orthosilicate (TMOS, Sigma Aldrich), 1.50 mL of deionized water and 0.40 mL of 0.04 M HCl based on an earlier literature [7]. The mixture was sonicated for 30 minutes to obtain the sol solution. The solution was shaken thoroughly and then sonicated for 30 minutes to fully mix the organic and inorganic phase. A sol solution was obtained after sonication.

A 0.02 M phosphate buffer (pH 8.0) was also prepared. Saturated solutions of the enzymes (5 mg in 1 mL buffer) were dissolved in 0.02 M phosphate buffer solution (pH 8.0). Equal volume (0.75 mL) of the sol solution and enzyme solution were mixed in polystyrene cuvette. Gelation was obtained within a minute. A blank was also prepared using just the phosphate buffer solution instead of the enzyme solution.

The formed gels or xerogels were stored at room temperature. The mass of the xerogels were obtained every day. The absorbance and emission spectra of the xerogels containing enzymes were obtained at different aging times (8,15, and 21 days-old). The absorbance was obtained using a Jasco V-570 spectrophotometer (Easton, MD). The emission spectra on the other hand were acquired on a FluoroMax-4 Spectrofluorometer (Horiba Jobin Yvon Inc., Edison, NJ). The sample was excited at its 280 nm to collect the emission scan from 290 to 500 nm with monochromator slits of 5 and 5 nm for the excitation and emission, respectively. In addition, the absorbance and emission spectra of the enzymes in solution was also obtained for comparison with that in xerogels.

Results and Discussion

Mass of Xerogels Upon Aging

The mass of xerogels decreased by 55-60% after 10 days of aging and by 64-75% after 20 days (Figure 1). The greatest loss in mass took place within the first five days, during which 44-53% reduction was observed. The xerogel containing glucose oxidase showed the highest reduction

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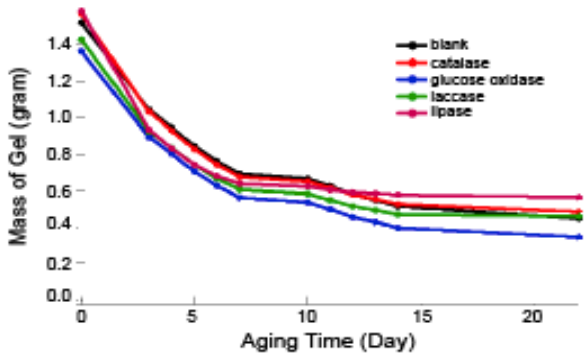

Figure 1: Mass of xerogels upon aging.

in mass of xerogels upon aging. The main reason for this decrease in mass is the removal of byproducts of the sol-gel process, including water and alcohol, by natural evaporation. During this drying process, a significant amount of shrinkage and densification was observed; the polymer network formed allowed for the immobilization of the enzyme. The shrinkage of the gel resulted in an increased concentration of enzymes within the xerogel (Figure 1).

Spectrochemical properties

Solution: The relative absorbance of enzymes in solution is shown in Figure 2. All enzymes have an absorbance around 270-280 nm, as typically observed in most protein samples. The peak at this region comes from the presence of aromatic amino acids (tryptophan, tyrosine and phenylalanine) in these enzymes [8]. It is also expected that another peak at around 200-220 nm will be observed. This is due to the electronic transitions of the peptide backbone itself [9-10]. However, due to the high concentration of the samples (with the exception of glucose oxidase), this was not observed. Instead, there is a broad peak stretching from the 280 nm region.

In addition, extra peaks can be observed in each of the enzymes. For the catalase, a band absorption peak at about 404 nm corresponds to the Soret absorption band belonging to $\pi \rightarrow \pi^*$ transitions of hemetoporphyrin [11]. This should also be observed in laccase. Glucose oxidase has peaks at 360 and 470 nm, which is typical for a flavoprotein. Lipase also has peaks at 360 nm and 410 nm. The absorbance of the enzymes encapsulated in the xerogels was not obtained due to the opaque nature of the resulting gels.

The steady state emission spectra of all enzymes are shown in Figure 2. These spectra were obtained using an excitation wavelength of 280 nm. For glucose oxidase, laccase and lipase, the emission max was observed at 350 nm, while the emission max for catalase was observed at 340 nm. A smaller peak was also observed at the 450 nm region for laccase (Figure 2).

Xerogels: Catalase is a heme protein belonging to the class of oxidoreductases, which can be used to catalyze the reduction of hydrogen peroxide into oxygen and water without the formation of free radicals [12]. As a protective enzyme, it is responsible for the degradation of hydrogen peroxide (H_2O) into molecular oxygen (O_2) and H2O in vivo [13]. Upon encapsulation, there is a red shift in the emission max of the catalase until day 15 (Figure 3). By 22 days, the emission max is similar to that of the solution. In addition, there is broadening of the peak with aging . This can be attributed to a higher concentration of the enzyme due to the shrinking of the xerogel. Lastly, a peak around 450 nm was observed after 15 days of aging and became more prominent by 22 days.

Glucose oxidase on the other hand, is a very stable enzyme and a glycoprotein [14-15]. It is a well known homo-dimer composed of two identical 80-kDa subunits and two non-covalently bound flavin adenine dinucleotides complexes (FAD). Although there are two fluorophores in glucose oxidase, emission scans were only obtained for aromatic amino acids. There is a blue shift of around 10 nm from the 350 nm emission max upon sol-gel encapsulation (Figure 4). Widening of the peak was observed between 8 and 15 days of aging. (Figure 3 and 4)

Laccase belongs to a group of enzymes called blue copper oxidases that catalytically oxidize phenols [16] or chlorinated biphenyls with a four-electron reduction of O_2 to H_2O. Upon encapsulation, laccase showed an increase of a peak at the 450 nm region which is higher than the peak observed in the 350 nm region (Figure 5). There is an observed red shift of the 450 nm peak between 8 and 15 days of aging.

Lipase is the enzyme that is responsible for the hydrolysis of fats/lipids. It is an important enzyme with a broad variety of applications in the food industry, fine chemistry, and in the pharmaceutical

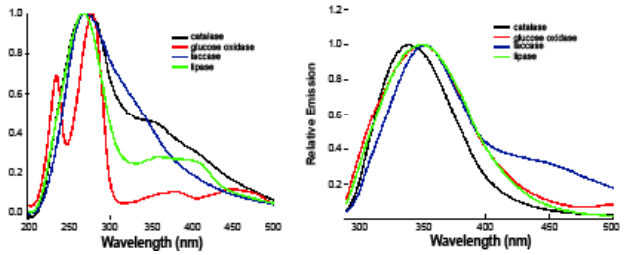

Figure 2: Relative absorbance (left) and emission (right) of enzymes in solution.

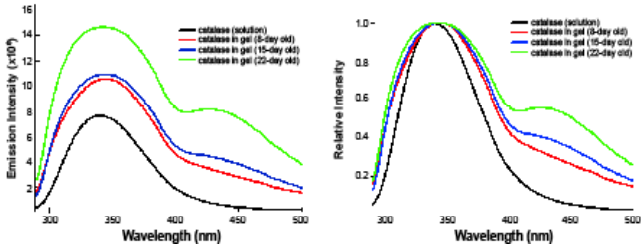

Figure 3: Emission and relative emission of catalase in solution and in xerogel.

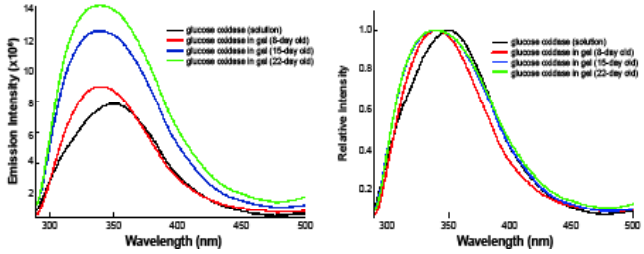

Figure 4: Emission and relative emission of glucose oxidase in solution and in xerogel.

industry due to the multiplicity of reactions it catalyzes [17]. Upon encapsulation, lipase showed the appearance of a shoulder peak at 300 nm and another peak at 440 nm by 15 days of aging (Figure 5).

The shift and broadening of the peaks that is observed upon encapsulation of enzymes in xerogel indicates possible changes in conformation. It has been reported that the entrapment of a protein in a sol-gel derived glass may result in significant conformational changes that are consistent with partial denaturation [18]. The changes of protein conformation and dynamic motion inside the gel are caused by interactions of the molecule with silica surface inside the pores and different microenvironment. The shrinkage of the gel during the condensation drying process may also cause denaturation of enzymes. Lastly, the released alcohols during the hydrolysis of silicon alkoxide can also inactivate enzymes (Figure 6).

The properties of the enzyme molecule inside the gel are altered due to different conformation. This change in conformation may lead to denaturation of the protein, restriction of molecule motion, and lower accessibility of the entrapped enzyme by the substrate [19,20]. The stability of the enzyme in the silica gel can either decrease or increase depending on the nature of protein and composition of the gel [19]. For instance, apomyoglobin was reported to unfold upon encapsulation [21].

The broadening of spectra have been reported before in glucose oxidase encapsulated in silica gel [22,23]. Both reported that the tryptophan fluorescence suggests that the conformation of the GOD molecule entrapped in silica gel is different than that of the enzyme in aqueous solution. The presence of alcohol produced during hydrolysis is not the only reason for these changes; the proportional symmetry of the broadening suggests that it may also due to the increase in concentration of the encapsulated enzymes upon shrinking of the xerogels.

The main reason for the observed peak shifts, such as the red shift for catalase and laccase and the blue shift for glucose oxidase, is the polarity of the environment around tryptophan residues. The emission spectrum of R-chymotrypsin reported a slight red-shift when encapsulated in silica gel. This shift likely reflects subtle changes in the polarity of the external solvent that perturbs the tryptophan moieties [18]. It is also possible that this is the cause in the appearance of emission peaks in some of the enzymes.

Conclusion

The spectrochemical properties of four enzymes encapsulated in xerogels were determined and compared with that of the enzymes in solution. Based on the emission profile, each enzyme showed distinct peak shifts, appearance of new peaks, and broadening of the spectra upon encapsulation. This is consistent with the changes in the conformation of the enzymes that occur as a result of the sol-gel process.

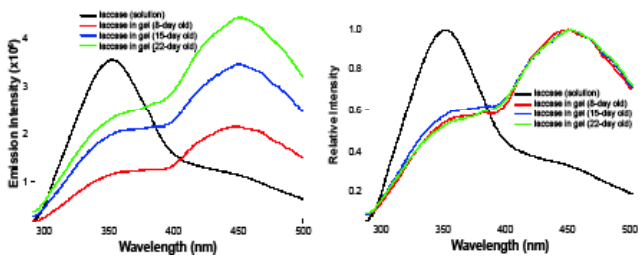

Figure 5: Emission and relative emission of laccase in solution and in xerogel.

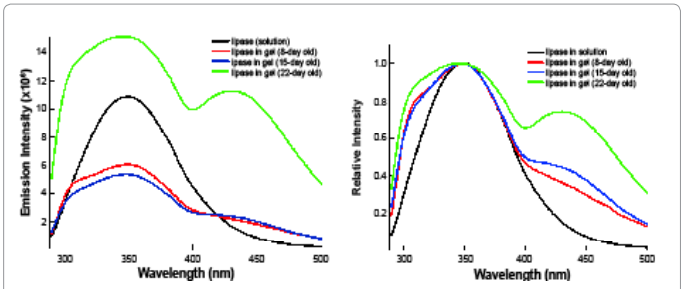

Figure 6: Emission and relative emission of lipase in solution and in xerogel.

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