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Tryptophan and Proteins Can Be Entrapped in UV Transmitting Glass

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It is demonstrated that tryptophan and proteins (parvalbumin and alcohol dehydrogenase) can be entrapped in silica glass. The silica glass is transparent in the UV spectral region, allowing for spectroscopic studies of immobilized proteins. The fluorescence spectra and phosphorescence spectra and lifetime of entrapped proteins resemble those observed for the molecules in aqueous solution.

KEY WORDS: Glass; tryptophan; phosphorescence.

INTRODUCTION

It was shown by Ellerby and co-workers that proteins could be encapsulated into silica gels without imposing significant alteration of the polypeptide structure [1]. Entrapment of proteins in glass offers a combination of uses in scientific research because (a) the encapsulated protein remains stable over time, (b) the transparency of the glass permits optical studies of immobilized proteins, and (c) glass can be made with selective porosity, allowing permeable substances to interact with the entrapped protein. Previous work has shown the value of these properties in the study of heme proteins encapsulated in sol-gels [2,3]. In these studies, use was made of the visible absorption spectrum of the heme; however, most proteins do not contain a chromophore that absorbs in the visible range. Of the 20 naturally occurring amino acids, tryptophan, tyrosine, and phenylalanine have absorption in the UV region, and to study their electronic spectra they must be in containers that transmit UV light. We show here that silica glass, prepared as described in the literature, is UV transparent, allowing for study of entrapped UV absorbing proteins. To demonstrate this, the luminescence properties of tryptophan, alcohol dehydrogenase, and parvalbumin were examined.

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MATERIALS AND METHODS

Materials

Tetramethyl orthosilicate (TMOS) was obtained from Aldrich Chemical Co. (Milwaukee, WI). L-Tryptophan was obtained from Fluka Biochemika (Buchs, Germany). Alcohol dehydrogenase was obtained from Sigma Chemical Co. (St. Louis, MO). Parvalbumin was prepared from frozen cod fillets, obtained at a local supermarket, as described previously [4]. Potassium phosphate buffer was used throughout; it had a pH of 6.0 and a concentration of 10 m*M*. Water was deionized and glass distilled.

The Sol-Gel Process

The silica sol was prepared by varying a published procedure [1]. A mixture containing 740 μl of TMOS, 200 μl of deionized water, and 2 μl of 40 m*M* HCl was sonicated for 30 s on an output control of 3 using a Branson sonifier. The sample warmed during sonification and it was cooled to approximately room temperature by putting it in a water bath. A clear, homogeneous sol was

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formed. Next 1 ml of 10 m*M* potassium phosphate buffer at pH 6.0 gelation and 1 ml of tryptophan or tryptophan-containing protein solutions in the same buffer were immediately added to the solution. In experiments where no protein or tryptophan was added, 2 ml of the buffer was used. The sol in both instances was quickly transferred into plastic cuvettes (10 × 10 × 48 mm). Polymerization was rapid and the sol set in the cuvette in less than 6 min. Initially water or buffer was added to the top surface of the glass and parafilm was placed around the top of the cuvette to aid in the aging process. After 1 day the water and methanol that had accumulated as the glass slowly condensed were removed from the sample and, again, each day thereafter. After 2–3 days the glass had condensed so that it no longer adhered to the surface of the plastic cuvette. At this time a pin hole was pierced in the parafilm to allow slow drying. Slow drying aids in the glass annealing process [5].

Spectroscopic Measurements

Steady-state absorption spectra were obtained with a Model 200 Perkin–Elmer spectrometer. Steady-state fluorescence and phosphorescence spectra were obtained on a Perkin–Elmer LS-5 luminescence spectrometer. The solid piece of glass was placed in the cell holder for some measurements. However, because the surface of the prepared glass was not optically smooth, it scattered light. To avoid light scattering problems, the solid glass piece was placed in water or buffer in a quartz cuvette for most spectroscopic measurements.

Oxygen Removal from Samples

The sol-gel was removed from its plastic cuvette and placed into a quartz cuvette which, contained a small volume of a solution made up of glucose/glucose oxidase and catalase in potassium phosphate buffer as described previously [6]. The cuvette was then closed with a quartz stopper and the top was wrapped with parafilm. The sample was allowed to sit at room temperature for 24 h.

RESULTS

Absorption and Fluorescence of Tryptophan in Glass

A comparison of the UV absorption of Trp encapsulated in glass and in solvent was performed. As shown in Fig. 1, the absorption spectra of Trp in solvent and in glass showed the same general features.

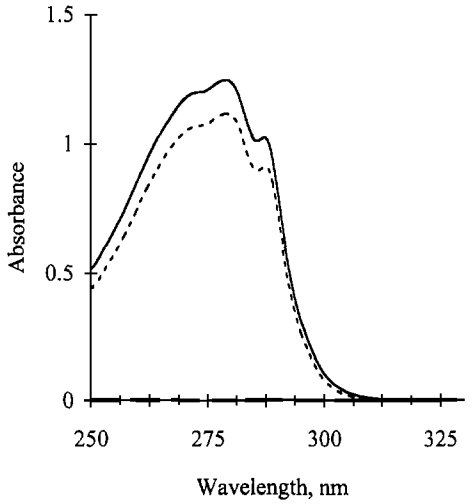

Fig. 1. UV absorption spectra of tryptophan in 10 m*M* potassium phosphate buffer (KP_i) at pH 6.0 (solid line) and in glass (dashed line). The glass was aged for 3 days. Absorption spectrum of the control glass (i.e., no added tryptophan) is shown as the dash–dot line.

The fluorescence spectrum of the encapsulated molecule relative to phosphate buffer is shown in Fig. 2.

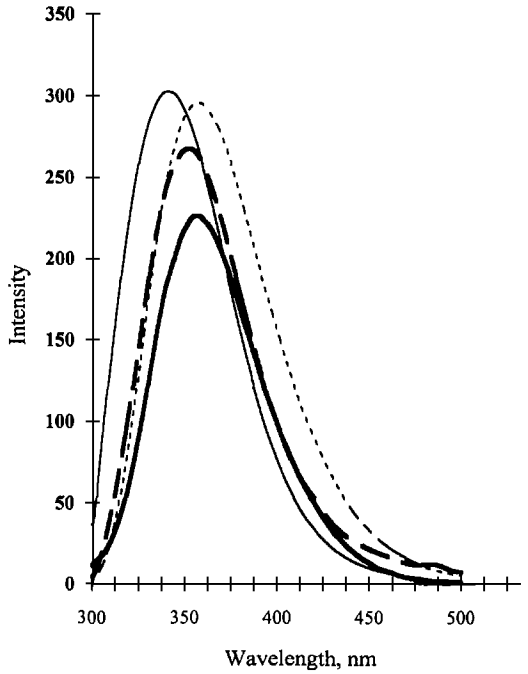

Fig. 2. Fluorescence emission spectra of tryptophan. The tryptophan was in 10 m*M* KP_i buffer (light dotted line), in methanol (light dashed line), or in glass aged for 1 day (dark dashed line) or for 4 days (dark solid line). Excitation λ, 280 nm; excitation bandpass, 10 nm; emission bandpass, 3 nm.

The emission maximum changed to a shorter wavelength initially after the glass was formed (dark dashed line; day 1) but shifted to a longer wavelength after further aging of the glass (dark solid line; day 4). The emission maximum for Trp in the sol-gel on day 4 is close to that observed for Trp in phosphate buffer (light dashed line). In Fig. 2 the emission of Trp in methanol is also shown (light solid line). As is well known [7] and also shown in Fig. 2, the emission of Trp is blue shifted in methanol relative to water. Since methanol is a product of the hydrolysis of TMOS, we consider that the likely explanation for the blue shift at the beginning of silica polymerization is that Trp is exposed to methanol. As aging occurs, methanol is lost, and the Trp fluorescence resembles that seen in a purely aqueous solution.

Protein Stabilization in Glass

Fluorescence emission spectra of alcohol dehydrogenase are shown in Fig. 3. The emission spectral shape remained constant over time. Unlike Trp in solution, the Trp in the protein is protected by the polypeptide chain and, therefore, less likely to be sensitive to solution effects. There was, however, an apparent decrease in fluorescence intensity. We considered the possibility that protein was being lost from the glass over time. This proved not to be the case since the absorbance did not change. A more likely possibility is more trivial: that the decrease in intensity was a result of shrinkage of the glass, which, using our instrument, would result in less sample being excited.

The fluorescence emission of parvalbumin encapsulated in the glass is shown in Fig. 4. The spectral shape and position are characteristic of Ca-bound parvalbumin [8,9].

Sol-Gel Porosity

At stages of aging between 1 and 8 days none of the sol-gel samples were seen to exhibit phosphorescence from tryptophan. It was suspected either that phosphorescence was being quenched by oxygen molecules that were entrapped in the glass lattice or that the glass was permeable to O_2. To test these possibilities, a sol-gel sample containing parvalbumin was immersed in the enzymatic deoxygenating solution described above. Within 24 h phosphorescence was detected (Fig. 5). The intensity decreased with an increase in delay time, consistent with the ~4- to 5-ms phosphorescence lifetime observed for parvalbumin in deoxygenated aqueous solution [4].

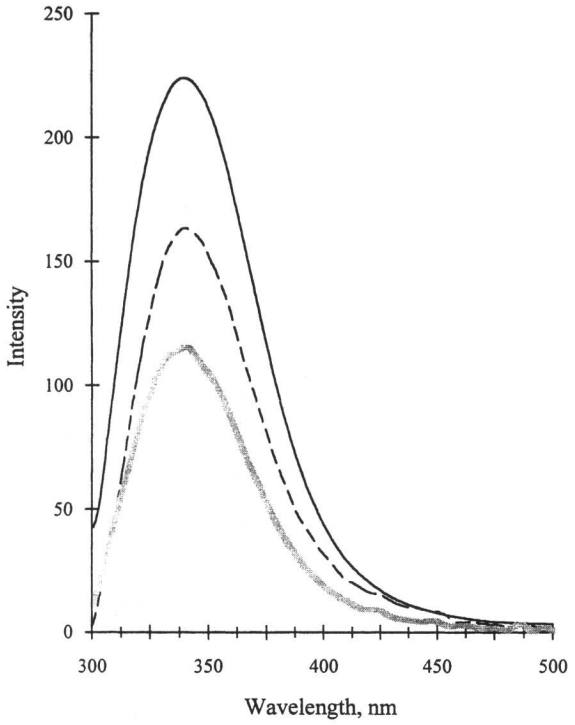

Fig. 3. Fluorescence emission spectra of alcohol dehydrogenase. Excitation λ, 280 nm. Solid line: 4 mg of protein/ml of 10 m*M* KP_i, at pH 6.0. Dashed line, alcohol dehydrogenase in glass aged for 1 day; shaded line, the same, aged for 4 days.

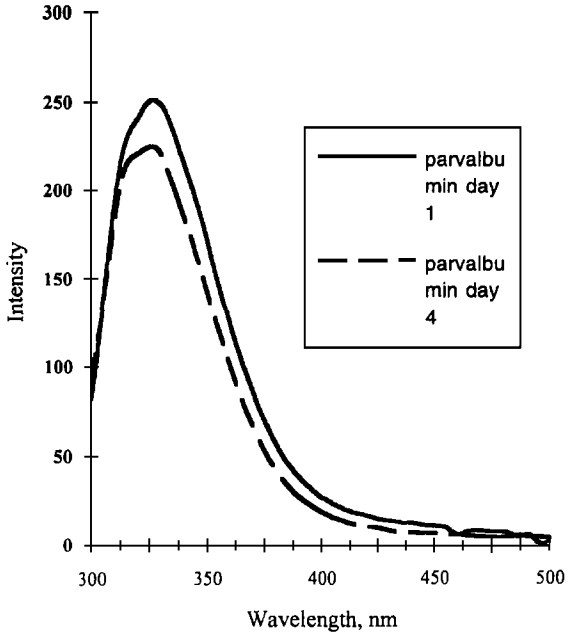

Fig. 4. Fluorescence emission spectra of parvalbumin. Excitation λ, 280 nm. Samples contained 4 mg of protein/ml of 10 m*M* KP_i, at pH 6.0 in the glass. Aging times are indicated.

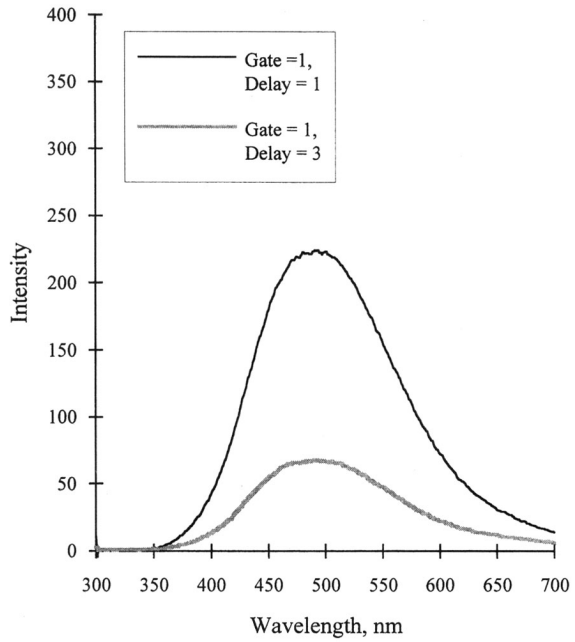

Fig. 5. Phosphorescence emission spectra of parvalbumin in glass after deoxygenating the sample. Excitation λ, 280 nm; gate time, 1.0 ms; delay times, 1 ms (solid line) and 3 ms (dashed line). Samples contained 4 mg of protein/ml of 10 m*M* KP_i at pH 6.0. Glass was aged for 8 days.

DISCUSSION

From the experimental point of view there are some advantages to immobilize proteins. For instance, it is often of interest to separate internal motions versus motions of the whole molecule. With immobilization in a glass the latter motion could be suppressed, while internal motions might not be. In energy transfer experiments, interpretation is easier when there is a randomly oriented, nonmobile sample than for rapidly rotating donors and acceptor. Detection of the optically excited triplet state by electron paramagnetic resonance requires immobilization since rotational motion would average the signal to zero. For the latter use, immobilization of spin probes and study of sol-gel glasses by EPR have already been achieved [10].

Immobilized proteins have additional interest in that they can be used as catalysts [11]. Such catalytic systems are readily adaptable for use as biosensors. The significant finding of our work is that the glass prepared from acid catalysis of TMOS results in a product that is transparent in the UV region. Since many enzymes have conformational-induced changes in tryptophan fluorescence, this greatly increases the possibilities of biosensors with optical detection.

In the glass as prepared here phosphorescence of Trp was seen only when the sample was deoxygenated. Due to its long lifetime, Trp phosphorescence is very sensitive to the presence of O_2 [12]. Since phosphorescence could be seen when O_2 was removed from the surrounding solution, O_2 appears to diffuse quite readily through the glass. Similarly prepared glasses were permeable to dithionite and CO [1]. Since glasses can be prepared with specific permeability to size or specific small ions, glass-immobilized proteins have additional attractiveness for use as biosensors.

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