Selective Intake and Release of Proteins by Organically-Modified Silica Sol−Gels

Mukti S. Rao and Bakul C. Dave*

Department of Chemistry and Biochemistry
Southern Illinois University at Carbondale
Carbondale, Illinois 62901-4409

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Selectivity at the molecular level is a key factor in recognition, sensing, and separation phenomena.[1−7] In this paper, we describe the synthesis and properties of a novel sol−gel-derived material that is capable of selective interactions with proteins. These materials were prepared by hydrolysis of bis[3-(trimethoxysilyl)-propyl]ethylenediamine (enTMOS) precursor. In these glasses, the porosity is modified to such an extent that large biomolecules can freely diffuse in and out of the bulk of the material. Consequently, intake and release of proteins is possible. In addition, these sol−gels exhibit protein-selective behavior with globular heme proteins such as ferricytochrome *c* (cyt *c*), myoglobin (Mb), and hemoglobin (Hb). Monoliths of enTMOS sol−gels, when exposed to a mixture of cyt *c* (MW ≈ 12400) and Mb (MW ≈ 16900), show an affinity for Mb and preferentially entrap Mb while leaving cyt *c* in the outside solution. The behavior of the porous gels when exposed to an equimolar mixture of cyt *c* and Hb (MW ≈ 64000) is particularly remarkable in that a preferential selectivity for Hb is observed. On the other hand, sol−gels containing a mixture of encapsulated cyt *c* and Mb (or cyt *c* and Hb) exclusively release cyt *c*. The overall processes occurring between enTMOS glass and heme proteins are qualitatively depicted in Scheme 1. An important distinction to be made is that the interactions with these proteins are not limited to the surface but occur throughout the bulk of the monolith.

The enTMOS sol−gels were prepared by treating the precursor with an equal volume of water. Typical preparations involve mixing 1 mL of enTMOS (Gelest, Inc. Tullytown, PA) with 1 mL of water in a polystyrene cuvette to obtain bulk monolithic pieces. The gelation times are on the order of 1−2 min. The freshly formed gels are allowed to age for 1−2 days. The aged sol−gels are solid state, optically transparent, rigid, and mechanically stable materials. Encapsulation of proteins in the enTMOS sol−gels can be achieved by substituting an aqueous solution of a protein (or a mixture of proteins) in place of water.

The intake and release of heme proteins was studied by using aged monolithic gels with approximate dimensions of 4 cm × 1 cm × 0.5 cm. For monitoring the intake of proteins, pristine enTMOS sol−gels were immersed in 2 mL of a solution mixture of proteins. The concentration of each protein in the aqueous mixture was 0.125 mM. The release of proteins was evaluated with sol−gels containing a mixture of proteins such that the concentration of each protein in the gel was 0.0625 mM. The use of heme containing proteins facilitates the application of optical spectroscopy to monitor the intake and release processes.

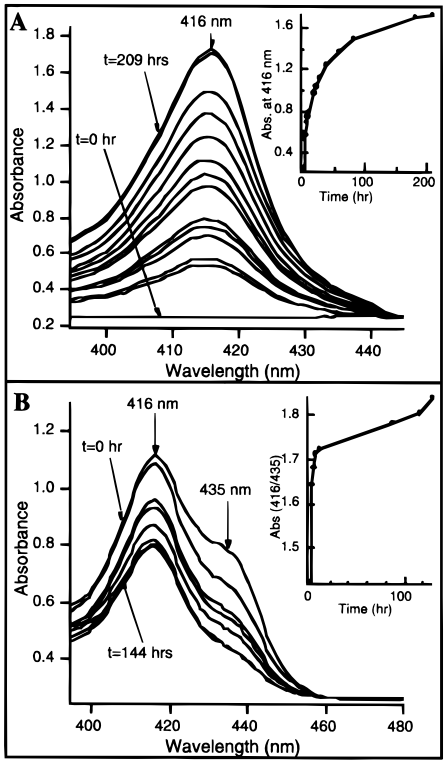
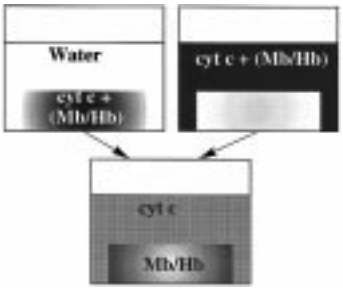


Figure 1. Intake/release behavior of enTMOS sol−gels with a mixture of cyt *c* and Mb. (A) Absorption spectra of contact solution showing time-dependent release of cyt *c* from a sol−gel containing an equimolar (0.0625 mM each) mixture of cyt *c* and Mb. Inset shows the time profile of intensity at 416 nm. (B) Absorption spectra of contact solution (aqueous mixture of cyt *c* and Mb, initial concentration 0.125 mM each) showing time-dependent intake of proteins by enTMOS sol−gels. Inset shows temporal variation of the ratio of intensities at 416 nm versus 435 nm.

Scheme 1



The Soret bands of cyt *c*, Mb, and Hb overlap in the oxidized form; however, in the reduced form they are quite distinct (λ_{max}: cyt *c*, 416 nm; Mb, 435 nm; Hb, 430 nm). The intensities of these bands were used to monitor time-dependent changes in relative concentrations of proteins in the contact solution. Absorption spectra of the contact solution were measured using 25 μL aliquots diluted with 0.5 mL of 0.1 M phosphate buffer (pH 7) and treated with sodium dithionite to obtain the reduced proteins. It is important to note that the intake/release experiments were performed with ferricytochrome *c*, metmyoglobin, and methemoglobin, whereas the optical measurements were obtained with the dithionite-reduced samples.

The intake/release behavior of enTMOS sol−gels with a mixture of cyt *c* and Mb is shown in Figure 1. When sol−gels containing an equimolar mixture of cyt *c* and Mb are exposed to water, a preferential release of cyt *c* is observed over time as

* To whom correspondence should be addressed.

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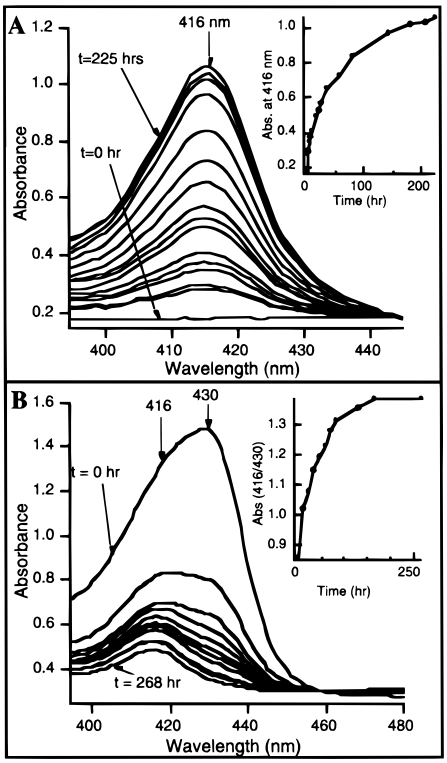

Figure 2. Intake/release behavior of enTMOS sol−gels with a mixture of cyt *c* and Hb. (A) Absorption spectra of contact solution showing time-dependent release of cyt *c* from a sol−gel containing an equimolar (0.0625 mM each) mixture of cyt *c* and Hb. Inset shows the time profile of intensity at 416 nm. (B) Absorption spectra of contact solution (aqueous mixture of cyt *c* and Hb, initial concentration 0.125 mM each) showing time-dependent intake of proteins by enTMOS sol−gels. Inset shows the time profile of changes in the ratio of intensities at 416 nm versus 430 nm.

indicated by an increase in peak intensity at 416 nm in the absorption spectra of the contact solution (Figure 1A). The absence of a band at 435 nm suggests that Mb is totally retained in the sol−gel. On the other hand, when plain enTMOS sol−gels are immersed in an equimolar mixture of cyt *c* and Mb, the sol−gels preferentially entrap Mb, leaving an excess of cyt *c* in the outside solution (Figure 1B). The ratio of intensities at 416 nm versus 435 nm is taken as a measure of relative concentration of cyt *c* with respect to Mb. Thus, the concentration of Mb in the external solution decreases sharply with time as shown by a change in the intensity ratio from an initial value of 1.42 to 1.64 in 1 h, and then to 1.73 in 9 h (Figure 1B, Inset). Gels kept in contact with the mixture of cyt *c* and Mb gradually turn dark-brown over an extended period. Visual inspection of deliberately fragmented monoliths reveals a uniformity of color within the entire sample, indicating that the entrapped protein molecules are distributed throughout the bulk of the gel.

The intake/release behavior of enTMOS sol−gels with a mixture of cyt *c* and Hb is shown in Figure 2. An exclusive release of cyt *c* is observed from gels containing the mixture (Figure 2A). On the other hand, when pristine sol−gels are exposed to an aqueous mixture of the proteins, a preferential intake of Hb (relative to cyt *c*) is observed (Figure 2B). Although a significant fraction of cyt *c* also diffuses into the gel, an affinity of the gel for Hb is clearly evident by the distinct change in the ratio of peak intensities at 416 nm versus 430 nm which varies from an initial value of 0.86 to 1.38 in 270 h (Figure 2B, Inset). The comparatively preferential intake of Hb, which is a much bigger molecule relative to cyt *c*, suggests that the protein affinity of the enTMOS gels is not size-selective.

The unique protein-selective behavior of enTMOS sol−gels derives from the distinct molecular composition of the precursor whose structure is given as

$$(CH_3O)_3Si(CH_2)_3NH(CH_2)_2NH(CH_2)_3Si(OCH_3)_3$$

The overall design strategy for using the precursor was based on several factors that facilitate selective interactions of enTMOS sol−gels with proteins. First, the use of an alkoxodisilane precursor with a long-chain spacer unit allows the formation of materials with enhanced porosity[8] for free mobility of proteins through the material.[9] Similar approaches taken by Shea and co-workers have established the feasibility of tailoring pore sizes by the use of a spacer group.[10] Second, the combination of amino groups and organic moieties in the spacer group provides a balance of hydrogen bonding and hydrophobic interactions, allowing preferential noncovalent interactions based on the structural composition of biomolecules. Third, the presence of ionizable amino groups ($pK_a \approx 11$) in the material furnishes opportunities for selective electrostatic interactions with proteins for charge-based selectivity. Inasmuch as the protein-selective behavior is intrinsically programmed into the enTMOS sol−gels, the experimental results are consistent with effectiveness of the molecular design approach.

Although at present a detailed understanding of the principal modes of interactions between proteins and enTMOS sol−gels remains to be established, it is apparent that the protein-selective behavior is a consequence of differential noncovalent interactions of proteins with the gel. Overall, the current results indicate that the enTMOS sol−gels show a reduced affinity for cyt *c* relative to Mb and Hb. The surface of cyt *c* is characterized by a predominance of charged hydrophilic residues on its surface.[11] Therefore, electrostatic effects are expected to play a role in selectivity. The amino groups present in the sol−gel material are possibly protonated under the conditions employed in this study such that the material should carry an overall positive charge. Cyt c is known to be positively charged[12] (an overall charge of +8) which leads us to believe that electrostatic repulsions between the positively charged pores of the gel and cyt *c* may be one of the factors responsible for the reduced affinity of the material for cyt *c*.

In conclusion, our results demonstrate the feasibility of selective intake and release of proteins using sol−gels with a molecularly designed porous structure. An important distinction of enTMOS sol−gels is that selectivity in these materials is achieved without molecular imprinting.[7] The enTMOS-derived materials incorporate an optimum balance of pore dimensions, hydrophobicity, and electrostatic charges, which allows a fine-tuning of noncovalent interactions between the sol−gel and a given protein, leading to selectivity. These materials may find potentially useful applications related to recognition, sensing, separation, and controlled release of proteins and enzymes.

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(8) The enlarged pore sizes of undoped enTMOS sol−gels relative to pure silica sol−gels obtained by hydrolysis of tetramethoxysilane (TMOS) are established by TEM and BET surface area measurements. TEM data shows an average pore diameter of 0.5 nm for TMOS sol−gels, whereas for enTMOS sol−gels enlarged pores with a diameter of 2 nm are observed. These observations are confirmed by surface area measurements which show a surface area of 288 m^2/g for TMOS xerogels and 577 m^2/g for enTMOS xerogels. Mazumdar, J.; Dave, B. C., unpublished results.

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