Hydrophobic, organically-modified silica gels enhance the secondary structure of encapsulated apomyoglobin†

Veronica A. Rocha and Daryl K. Eggers*

Received (in Cambridge, UK) 22nd November 2006, Accepted 19th December 2006
First published as an Advance Article on the web 11th January 2007
DOI: 10.1039/b617078a

Insertion of hydrophobic groups in a silica matrix, by addition of propyl- or trifluoropropyltrimethoxysilane, leads to a surprising increase in the helical content of apomyoglobin following encapsulation by the sol–gel technique.

Sol–gel entrapment of biological molecules in a silica glass material has been utilized for many applications including biosensor development and production of stable catalysts,[1] trapping of conformational intermediates,[2] high-throughput screening assays,[3] investigations on the effects of macromolecular crowding,[4] and even single-molecule studies.[5] Previously, apomyoglobin (apoMb), a model protein for folding studies, was found to have a highly unfolded conformation in wet-aged silica-glass samples prepared by an established protocol.[4] It was proposed that the silica surface has an unfavorable effect on the structure and properties of confined water in the pores of the glass and that these altered properties lead to the unfolded state of apoMb.[4b] With this hypothesis in mind, we set out to examine the effects of modifying the silica surface with functional groups not found in the standard glass prepared from tetramethoxysilane.

When a hydrophobic alkyl group was incorporated into the silica matrix by addition of propyltrimethoxysilane to the standard sol–gel recipe, a dose-dependent increase in the helical content of apoMb was observed, as monitored by circular dichroism (CD) spectroscopy in the far-UV region (Fig. 1a). At a molar value of 12% propyl-modified silane relative to total silane, the ellipticity of encapsulated apoMb approaches the same value observed in dilute solution, indicating an increase in helical content and overall structure. This result was surprising to us because one would expect a more-hydrophobic environment to favor the unfolded state of the protein over the native, folded conformation.

Fluorinated silica aerogels, made by the sol–gel technique, have been demonstrated to be excellent materials for adsorbing nonpolar molecules from water.[6] Because organic fluorine is considered to be more hydrophobic than its non-fluorinated counterpart, we decided to examine the effects of trifluoropropyl-modified glasses on the structure of encapsulated apoMb. These glasses also yield a dose-dependent increase in the secondary structure of apoMb, and the gain in secondary structure is significantly greater than that obtained with the same molar quantity of propyltrimethoxysilane (Fig. 1b). For example, 4% of the trifluoropropyl reagent has approximately the same effect as 12% of the propyl reagent, and further increases in the fluoropropyl-group content lead to apoMb conformations that exceed the helicity of the protein observed in dilute solution. At a value of 12% trifluoropropyl incorporation, the mean residue molar ellipticity of encapsulated apoMb is $-22\,400^\circ$ cm^2 dmol^{-1}, whereas the ellipticity of the native state of the apoprotein, as defined in dilute solution, is approximately $-19\,000^\circ$ cm^2 dmol^{-1} at a wavelength of 222 nm. For comparison, the heme-bound holoprotein has a value near $-24\,000^\circ$ cm^2 dmol^{-1} at 222 nm.

We do not report any results for glasses made with higher percentages of alkyl-substituted silane reagents because modified glasses were found to lose optical transparency above a value of 14%, preventing analysis by far-UV CD.

In our previous studies with unmodified glasses, the unfolded state of encapsulated apoMb was found to be highly responsive to molar salt solutions in an order corresponding to the ranking of the Hofmeister series of ions.[4] When we examined the effect of 1.0 M potassium phosphate on the structure of apoMb in the

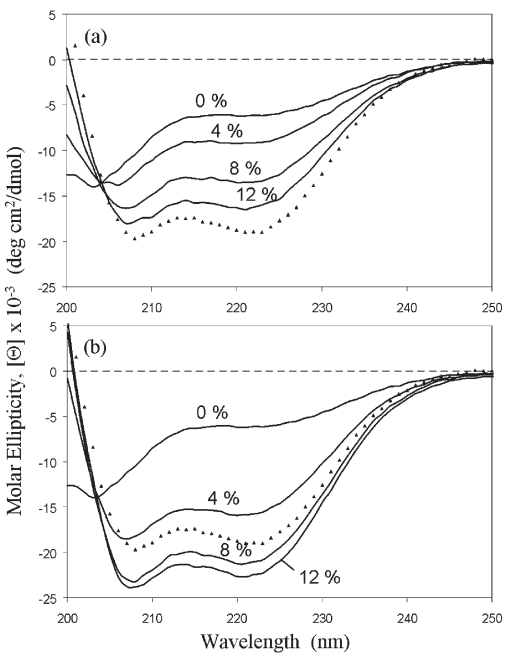

Fig. 1 Addition of (a) propyltrimethoxysilane or (b) trifluoropropyltrimethoxysilane increases the helicity of encapsulated apoMb. The molar percentage of mono-substituted silane reagent employed for each wet-aged glass is given next to the corresponding CD spectrum. In both panels, the spectrum of apoMb in dilute solution is given as a reference (···). All samples are in 10 mM potassium phosphate, pH 7.0.

Department of Chemistry, San José State University, San José, CA, USA 95192-0101. E-mail: deggers@science.sjsu.edu; Fax: (+1) 408 924 4945; Tel: (+1) 408 924 4960

† Electronic supplementary information (ESI) available: Materials and methods, response of glass-encapsulated apoMb to molar phosphate, and thermal stability of glass-encapsulated lysozyme and serum albumin. See DOI: 10.1039/b617078a

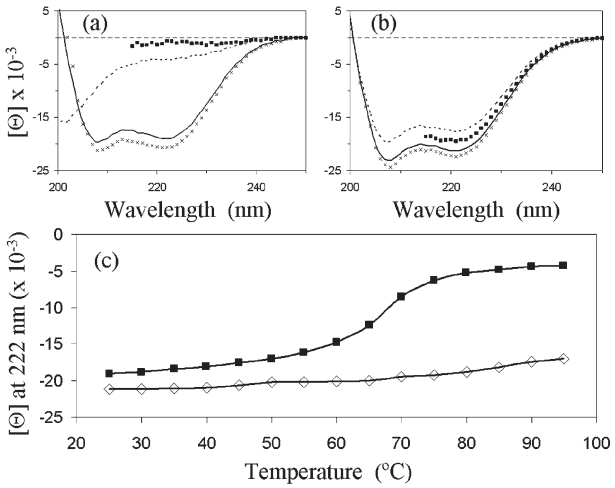

Fig. 2 Glass-entrapped apoMb is greatly stabilized against chemical and thermal denaturation. CD spectra of apoMb in (a) solution and (b) 8% trifluoropropyl-modified glass, in the presence of 10 mM potassium phosphate, pH 7.0 (—), 1.0 M potassium phosphate, pH 7.0 (×), 10 mM HCl (---), and 4.0 M guanidinium chloride with 10 mM phosphate, pH 7.0 (■). (c) Thermal denaturation curve for apoMb free in solution (■) and encapsulated in 8% trifluoropropyl-modified glass (◇), both in 10 mM potassium phosphate, pH 7.0.

propyl- and trifluoropropyl-modified glasses, a minor increase in ellipticity was observed, however, the favorable effects of high phosphate concentration and glass modification were not fully additive.† In the case of a glass made with 8% trifluoropropyl content, the gain in ellipticity is about the same as that observed in dilute solution when the phosphate concentration is increased 100 fold, from 10 mM to 1.0 M (Fig. 2a,b).

The stability of apoMb in hydrophobic glasses was tested by addition of chemical denaturants and by heating. In the presence of 10 mM HCl or 4 M guanidinium chloride, the secondary structure of apoMb is largely absent in dilute solution, as indicated by the decrease in molar ellipticity (Fig. 2a). In the hydrophobic glass, however, the ellipticity of apoMb was only modestly reduced, demonstrating that glass encapsulation provides outstanding resistance against acidic conditions or guanidinium chloride treatment (Fig. 2b). The thermal stability of glass-entrapped apoMb is equally impressive; upon heating the sample from 25 °C to 95 °C, only a slight decrease in ellipticity from $-21\,000$ to $-17\,000^{\circ}$ cm^2 dmol^{-1} was observed at a wavelength of 222 nm. By contrast, in dilute solution, apoMb proceeds to unfold through a broad transition with a midpoint near 66 °C (Fig. 2c). We conclude that the enhanced protein stability observed in the organically-modified glasses described here is equal to or better than that reported for proteins encapsulated in unmodified silica glasses.[4a]

To this date, hydrophobic, organically-modified sol–gel materials have been employed primarily for encapsulating proteins that associate with lipid interfaces or proteins that bind nonpolar ligands, such as lipases.[7] The increase in activity of lipases in hydrophobic silica glasses relative to unmodified glasses has been attributed, in part, to an increase in the partitioning and diffusion of nonpolar substrates from the aqueous phase into the glass matrix. The results presented here, using CD to assess the structure of the encapsulated protein, suggest that organically-modified glasses may also increase the fraction of properly-folded and functional enzymes in the pores of the glass. It should be noted that the previously-reported lipase studies typically used a much higher ratio of organically-modified silane, and the samples were air-dried to form xerogels, unlike the wet-aged glasses described in this work.

It is known that alcohols, in general, and trifluoroethanol (TFE) and hexafluoroisopropanol (HFIP), in particular, are potent inducers of helical structure in polypeptides. At high concentrations, TFE and HFIP have been shown to induce helices at the expense of tertiary structure.[4a] Because the trifluoropropyl group used in our study is a structural analog of TFE and HFIP, it follows that the observed increase in secondary structure of apoMb in trifluoropropyl-glasses may not correspond to a more native, properly-folded protein. To partially address this concern, the structure and stability of two other proteins, each known to have the same structure in the standard unmodified glass as in dilute solution, were examined in the hydrophobic glasses. Both chicken egg white lysozyme and human serum albumin retained their native structures after encapsulation in 8% fluoropropyl-modified glasses; there was no indication that these proteins became hyperhelical at the expense of tertiary structure.† Furthermore, encapsulated lysozyme and serum albumin exhibited remarkable thermal stabilities, similar to apoMb. In the case of lysozyme, the reversibility of thermal denaturation was better in the 8% trifluoropropyl-glass† than reported previously for the standard unmodified glass.[4a]

The 'polar hydrophobicity' of organic fluorine has been exploited in the design of enzyme inhibitors for medicinal chemistry[8] and, more recently, as a tool in protein engineering.[9] We hypothesize that the mechanism by which alcohols induce helical structure in polypeptides is the same mechanism by which hydrophobic, organically-modified glasses induce structure in apoMb. Although the physical basis of this phenomenon is still unclear, our data suggest that it is the hydrophobic component of alcohols, and not the hydroxyl group or the amphiphilic character, that induces helical structure in proteins because propyl- and trifluoropropyl-modified silica glasses have a similar effect on apoMb. Sol–gel encapsulation in organically-modified silica glass appears to be an interesting experimental system for probing the influence of interfacial chemistry on protein folding equilibria.

DKE is funded by the NIH through the MBRS-SCORE Program (grant #S06 GM008192). VAR is a recipient of an undergraduate training grant through the NIH MARC Program (grant #5 T34 GM008252).

Notes and references

1 (*a*) W. Jin and J. D. Brennan, *Anal. Chim. Acta*, 2002, **461**, 1; (*b*) A. C. Pierre, *Biocatal. Biotransform.*, 2004, **22**, 145.
2 (*a*) N. Shibayama and S. Saigo, *J. Mol. Biol.*, 1995, **251**, 203; (*b*) L. Ronda, S. Bruno, C. Viappiani, S. Abbruzzetti, A. Mozzarelli, K. C. Lowe and S. Bettati, *Protein Sci.*, 2006, **15**, 1961.
3 (*a*) C. B. Park and D. S. Clark, *Biotechnol. Bioeng.*, 2002, **78**, 229; (*b*) T. R. Besanger, Y. Chen, A. K. Deisingh, R. Hodgson, W. Jin, S. Mayer, M. A. Brook and J. D. Brennan, *Anal. Chem.*, 2003, **75**, 2382.
4 (*a*) D. K. Eggers and J. S. Valentine, *Protein Sci.*, 2001, **10**, 250; (*b*) D. K. Eggers and J. S. Valentine, *J. Mol. Biol.*, 2001, **314**, 911.
5 G. Baldini, F. Cannone and G. Chirico, *Science*, 2005, **309**, 1096.
6 T. M. Tillotson, K. G. Foster and J. G. Reynolds, *J. Non-Cryst. Solids*, 2004, **350**, 202.

7 (*a*) J. D. Brennan, J. S. Hartman, E. I. Ilnicki and M. Rakic, *Chem. Mater.*, 1999, **11**, 1853; (*b*) M. T. Reetz, P. Tielmann, W. Wiesenhöfer, W. Könen and A. Zonta, *Adv. Synth. Catal.*, 2003, **345**, 717; (*c*) G. Kuncova, J. Szilva, J. Hetflejs and S. Sabata, *J. Sol–Gel Sci. Technol.*, 2003, **26**, 1183; (*d*) H. El Rassy, S. Maury, P. Buisson and A. C. Pierre, *J. Non-Cryst. Solids*, 2004, **350**, 23; (*e*) P. Vidinha, V. Augusto, M. Almeida, I. Fonseca, A. Fidalgo, L. Ilharco, J. M. S. Cabral and S. Barreiros, *J. Biotechnol.*, 2006, **121**, 23.
8 J. C. Biffinger, H. W. Kim and S. G. DiMagno, *ChemBioChem*, 2004, **5**, 622.
9 C. Jäckel and B. Koksch, *Eur. J. Org. Chem.*, 2005, 4483.

