Use of CD Spectroscopy to Assess the Biocompatibility of Silica-based Materials

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Statement of Purpose: The general goal of this project is to develop new silica materials of high biocompatibility, as defined by enhanced protein structure and stability following encapsulation in a glass matrix. The sol–gel technique is employed to make organically-modified glasses, and circular dichroism spectroscopy (CD) is used to analyze changes in the structure of encapsulated model proteins as a function of surface chemistry.

Methods: New glasses were made using variations in a standard sol–gel recipe that employs tetramethoxysilane (TMOS) as the main precursor [1-3]. The sol was formed from mixtures of TMOS and monosubstituted alkoxy-silanes, $RSi(OCH_3)_3$, that feature different hydrophilic groups in the R-position. The modifying reagents were selected to include negatively-charged (-), positively-charged (+), and uncharged (Ø) hydrophilic groups, all from Gelest, Inc. Glass samples were cast as 1-mm thick sheets in a plastic cassette (Invitrogen). Model proteins for this study were apomyoglobin (apoMb), made by extracting the heme cofactor from horse heart myoglobin, and polylysine, both from Sigma-Aldrich. Changes in the structure of encapsulated proteins were monitored by CD analysis in the far-UV range (Aviv). As depicted in Fig.1, the resulting glass material may be envisioned as a highly porous matrix where only a small fraction of the pores are occupied by protein; two protein molecules are shown in Fig.1. In general, proteins are unable to diffuse out of the glass matrix, but water and small solutes may diffuse through the interconnected pores (white regions). The bulk solid occupies only ~15% of the total volume, and the average pore diameter is approximately 20 nm.

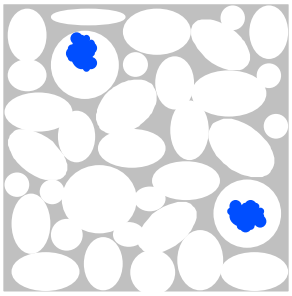

Figure 1. Cross-section of hypothetical glass wafer.

Results: ApoMb was encapsulated in a control glass (100% TMOS) and in all three hydrophilic glasses prepared with 10% of each modifying reagent. As seen in Fig.2, each of the modified glasses resulted in a more negative ellipticity value than the control glass, indicating an increase in the helical content of apoMb. The (+) glass gave the best result, but none of the glasses yielded a CD spectrum that matched the spectrum of the protein in dilute solution. Polylysine was also encapsulated in a control glass and in a modified glass containing 5% of both (-) and (+) ionic groups. In this case, the response of the polypeptide to a specific salt, $LiClO_4$, was analyzed.

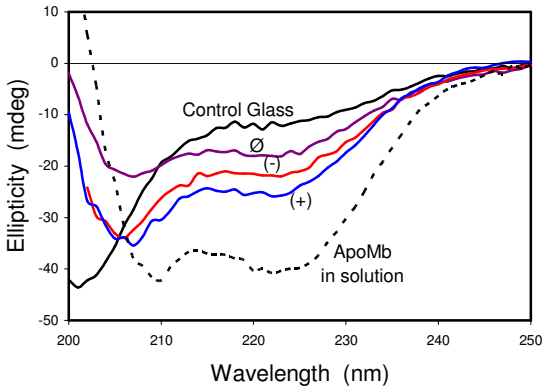

Figure 2. CD spectra of apoMb in 10%-modified glasses.

In solution, perchlorate is known to convert polylysine from a random coil to a stable helix structure at pH 7. As shown in Fig.3, polylysine has a spectrum that indicates a random coil in the control glass and changes very little upon addition of perchlorate (dashed line). In the modified glass, polylysine starts with more structure and becomes much more helical upon addition of $LiClO_4$.

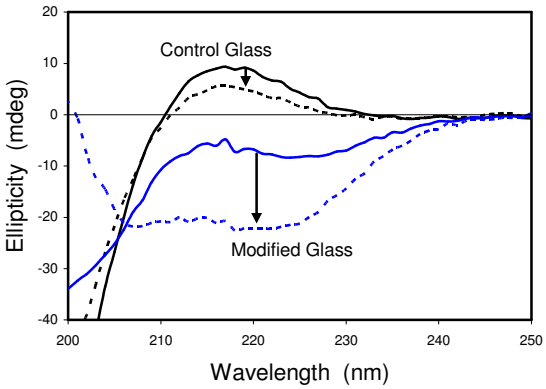

Figure 3. CD spectra of polylysine in a control and 10%-modified glass, before & after addition of 2M $LiClO_4$.

Conclusions: Organically-modified silica glasses can be made of suitable optical transparency for analysis by CD spectroscopy. In this study, both charged and uncharged hydrophilic groups were found to enhance the structure of apomyoglobin relative to an unmodified silica glass, but no condition fully restored the native structure of the protein as defined in dilute solution. The experiment with polylysine demonstrates that minor changes in glass chemistry can alter protein behavior in a direction that better mimics the properties of the protein in aqueous solution. Further research, combining two or more modifying reagents, may be needed to obtain the most biocompatible environment for protein encapsulation.

References:

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[2] Rocha VA. Chem Commun. 2007:1266-1268.
[3] Menaa B. Biomaterials. 2008;29:2710-2718.