The Use of Alkanethiols and Circular Dichroism to Monitor Conformational Changes in Adsorbed Proteins

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Introduction: Understanding protein-surface interactions is central to understanding and controlling cellular response to biomaterials. Circular dichroism (CD) provides an experimental means to monitor changes in the secondary structure of proteins in solution and those adsorbed on surfaces. Recent developments in g-factor analysis of CD data has removed some of the problems associated with adsorbed protein studies, and provides an accurate method for measuring adsorbed secondary structure.[1] By combining established CD techniques with engineered surfaces modified with alkanethiol self-assembled monolayers (SAMs), the effect of surface chemistry on the conformation of adsorbed proteins can be explored. The objective of this research was to develop methods to enable CD spectropolarimetry with g-factor analysis to be used to measure the change in the secondary structure of adsorbed proteins to alkanethiol SAM surfaces.

Materials and Methods: *Surface Preparation* – SAMs were formed by first depositing 50 Å each of Ti and Au on quartz substrates using electron beam deposition, which provide the adhesion layer for SAM assembly while remaining transparent. The slides were treated with 1-dodecanethiol (Sigma #471364), 11-mercapto-1-undecanol (Sigma #447528), 1-mercaptoundecanylamine (Prochimia #FT 02A.11-1), and 12-mercaptododecanoic acid (Prochimia #FT 01.11-1) resulting in a variety of SAM surfaces. Contact angle measurements were taken to verify that the surfaces demonstrated the expected degree of hydrophilicity.

Circular Dichroism – A Jasco J-810 CD instrument was used for all experiments. Porcine albumin (Sigma A1173) was dissolved in phosphate buffered saline (PBS) at a pH of 7.4. All measurements for solution structure were performed in a 1 cm quartz cell. For adhesion studies, a Hellma cell with a path length of 2 cm was used, which allows for the insertion of quartz slides. For adhesion studies, albumin solutions of 1 mg/ml were used, and surfaces were exposed overnight to equilibrate the adsorbed proteins.

g-Factor Analysis – The ratio of the difference between left and right absorbance of circularly polarized light to the absorbance at each wavelength results in a unitless g-factor. Current spectropolarimeters are able to simultaneously record these measurements, and the spectrum analysis software provided with the instrument readily calculates the g-factor from the recorded data.

Secondary Structure Prediction –The percent of alpha helix content was calculated by the CONTIN and SELCON3 CD spectral deconvolution algorithms using appropriate g-factor reference sets. The reference sets needed for such calculations were obtained thanks to the assistance of Dr. Robin L. Garrell, UCLA.

Results and Discussion: From Figure 1, the ability of g-factor analysis to normalize the data is readily demonstrated. While CD values can vary greatly (based on concentration, path length, etc.), unitless g-factor values are based only on secondary structure effects. The SAM surfaces exhibited clear signals, demonstrating the feasibility of the use of these types of surfaces for this and future studies. Table 1 below shows the structural changes of albumin that were measured following adhesion.

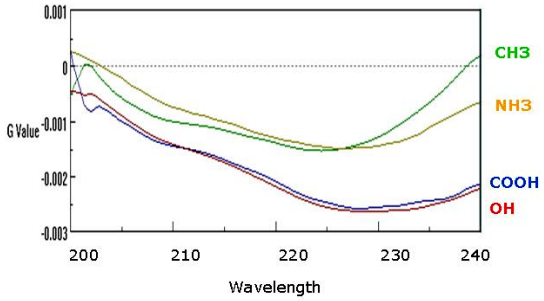

Figure 1: g-Factor and CD curves for Albumin on various surfaces.

Table 1. α-Helix Content from g-factor Analysis Albumin

Solution	Quartz Slide	NH_3	CH3	OH	COOH
53.13 % (+/- 5.96)	46 % (+/- 3.82)	22.2 % (+/- 1.45)	20.93 % (+/- 1.76)	36.7 % (+/- 2.56)	32.86 % (+/- 2.15)

For all data, n = 3. The mean plus/minus the standard deviation is shown.

Conclusions: The use of SAMs formed on gold-coated quartz slides in CD studies is a feasible method of designing model surfaces that can be used to study protein-surface interactions as a function of surface chemistry. CD with g-Factor analysis offers a reliable method for determining the secondary structure of adsorbed proteins. Using this technique, it is possible to quantify the degree of structural change that occurs at the protein-biomaterial interface.

Future Work: By probing at wavelengths below 200nm, it is possible to extract significant data regarding beta sheet and turn content as well as alpha helix. Preliminary results using fibrinogen (adsorbed on quartz slides) has shown a significant decrease in alpha helix (15 %) content with an increase in beta sheet (10 %). This relationship has be observed in literature.[2] We hope to examine this on surfaces of varying functionality, and determine the correlation between these structural changes and platelet adhesion.

References: [1] Baker B, Garrell R. Faraday Discuss 2004;126:209-222. [2]Yongli C, et al. J Colloid Interface Sci 1999;214(1):38-45.