Influence of Chemical Additives on the Structure and Biological Activity of Proteins Pre-adsorbed on Material Surfaces Using an Improved Circular Dichroism Methodology

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Statement of Purpose: The use of chemical additves to disrupt the structure and thereby deactivate adsorbed proteins on surfaces is one of the strategies that could be employed for passivating bioactive surfaces. But, lack in fundamental understanding of the mechanisms underlying the interaction of chemical additves with the structure of adsorbed proteins have limtited the effective application of this strategy. The objective of current study was thus to quantitatively assess the influence of chemical additives on the structure and bioactivity of adsorbed proteins

Materials and Methods:

Protein and Surfaces: Hen egg white lysozyme (HEWL) and Ribonuclease-A (RNase-A) dissolved in 10 mM potassium phosphate buffer (PPB, pH 7.4) was adsorbed on fused silica glass (glass), high density polyethylene (HDPE), and poly(methyl-methacrylate) (PMMA).

Protein Adsorption and Chemical Treatment: Proteins were adsorbed onto each adsorbent material from 0.03 mg/mL and 1 mg/mL of protein concentrations for 2 h to saturate the surface, after which surfaces were rinsed under running water and incubated at room temperature for 24 h in pure PPB to equilibrate the adsorbed proteins. Each of the adsorbent surfaces was then treated with the respective solutions of sodium dodecyl sulfate (SDS; 0.5% (w/v), Sigma, 75746), octyl glucoside (Octyl; 30 mM, Sigma O8001), 3-[(3- Cholamidopropyl) dimethyl ammonio] -1-propane sulfonate (CHAPS, 20 mM, Sigma C3023), urea (8 M, Fisher Scientific, U15500) and chloride salts of guanidinium (GdmHCl; 6 M, Sigma, G3272) for 17 h. The chaotropic agents like urea and GdmHCl were used as models of non-ionic and ionic interacting polar molecules, respectively, while detergents like SDS, Octyl and CHAPS were used as anionic, non-ionic and zwitterionic amphiphillic models above their critical micelle concentrations, respectively. No pH adjustments were made in any of the solutions.

CD Spectroscopy: CD was used to determine the structure of proteins in solution, the amount of protein adsorbed on each surface, and the adsorption induced conformational changes in proteins on each of the three material surfaces before and after treatment with chemical additives. The solution structure of the proteins was determined in quartz cuvettes (Starna Cells) while the structure of the adsorbed proteins was determined using a custom-designed cuvette [1, 2] over 230 nm-300 nm range. The fractional helical content (FH) of adsorbed proteins were quantified using an empirical relationship (eq 1) obtained from the slope (A) of the 230-240 nm of the CD spectra, as the strong background absorbance severely limits the application of the conventional quantification techniques [3].

$$FH = 0.000514*A + 0.00297 \quad \text{(eq 1)}$$

Bioactivity Assays: Turbidometric assays to monitor the enzymatic activity of HEWL and RNase-A were carried out using previously described methodology [2]. Briefly, the assays to determine the enzymatic bioactivity were done at pH 7.4 for a time period of 10 min for both the proteins by monitoring the decrease in absorbance at 300 nm (ΔA_{300}) and 450 nm (ΔA_{450}) for each of the enzymes when its acts on bioactive substrates of *Micrococcus lysodeikticus* (Sigma M3770) and ribonucleic acid (Baker's yeast, Sigma R6750), respectively. [2].

Results and Discussion: The influence of chemical additives on the structure was evaluated by estimating the helical content of adsorbed proteins using the 230-240 nm slope method. This technique enables us to accurately track the structural changes in adsorbed proteins by CD for a variety of solution conditions and surface chemistries in the presence of chemical additives with strong absorbance below 230 nm. These structural changes were subsequently used to evaluate its influence on the adsorbed state bioactivity of proteins.

The results shown in Figure 1a for PMMA following chemical treatment indicated that, though the additives like urea and GdmHCl induced almost complete loss in helical content of the proteins in solution (data not shown), these agents were ineffective in inducing significant disruption of the protein structure than the adsorbent surface. However, detergent molecules like SDS, CHAPS and Octyl which did not induce much helical changes in the proteins when in solution (data not shown), were found capable of inducing significant disruption of the adsorbed protein structure, especially when electrostatic interactions were involved. Additionally, it was also observed that the denaturation effects by each of the agents were more prominent in protein-systems that were adsorbed from low solution concentrations than high solution concentrations. The general loss in bioactivity of adsorbed enzymes post chemical treatment were consistent of that resulting from a loss in helical structure. (Fig 1b)

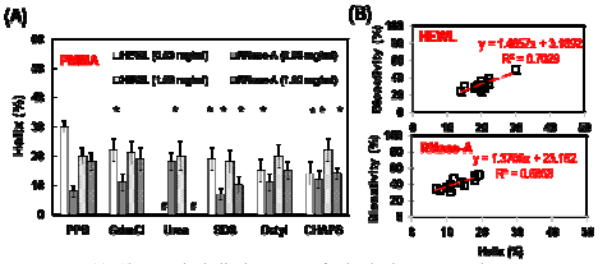

Figure 1. (a) Changes in helical content of adsorbed HEWL and RNase-A on PMMA surface as a function of chemical additives (N=3, mean ± 95% C.I.). '*' indicates significant change to the helical content of proteins when compared to the untreated condition in PPB. '#' refers to weak signal to noise ratio as a result of ≥ 85% removal of adsorbed protein. (b) The correlation between the bioactivity and helical content (%) of adsorbed HEWL and RNase-A on PMMA surfaces post chemical treatment.

Concluding Remarks In this study, we used 230-240 nm CD methodology to quantify the adsorbed structure of proteins in the presence of strongly absorbing chemical additives, and also assessed their effect on the bioactive state of proteins. Based on these results, it is evident that the molecular processes occurring in the solution and adsorbed phase of the proteins are different, and these interactions vary with the adsorption conditions and the type of chemical additives. Thus, with more fundamental insights, more effective strategies to passivate a wide range of bioactive surfaces by chemical agents could be devised.

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References: [1] Sivaraman et al, Langmuir. 2009, [2] Wei et al, Colloid Surface (2013) [3] Wei et al, BBA Proteins Proteom (2014).