Quantitative Measurement of Adsorption of pH Dependent Structures Adopted by BSA

A Comparative Study Between Dual Polarisation Interferometry and Neutron Reflection

Introduction

Farfield's ***Ana*****Light**® Dual Polarisation Interferometry (DPI) instrument enables unequivocal, high resolution measurement of mass, dimensions and density of immobilised biomolecules in real time. This application note demonstrates the power of ***Ana*****Light**® to quantitatively study pH dependent adsorption and conformational changes in proteins.

Results and Discussion

The surface adsorption behaviour of BSA ($0.1 mgml^{-1}$ in PBS) onto an unmodified ***Ana*****Chip™** was studied at pH3, pH5, and pH7. The ***Ana*****Chip™** surface was hydrophilic and negatively charged.

BSA has three principal domains with individual pH dependent net charges and an isoelectric point (IEP) around 4.8. **Figure 1** shows the different conformational isomers of BSA that form reversibly in solution at different pH values.

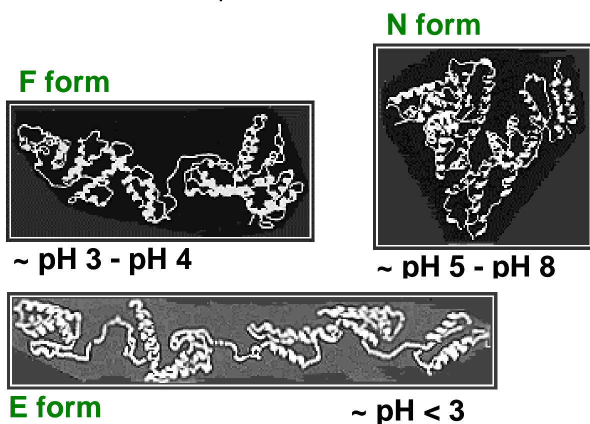

Figure 1: pH Dependent BSA isoforms – N (normal), F (fast) and E (expanded) – Occurring in Solution at pH5-8, pH3-4 and below pH3

Figure 2 shows how thickness, mass per unit area, and refractive index (RI) of the adsorbed BSA layer varied for the pH cycle 3-5-3. At pH3 a thin, dense layer formed as the predominantly positively charged and unfolded BSA attracted electrostatically to the ***Ana*****Chip™** surface. Adsorption increased as BSA at pH5 was added. With minimal net charge at pH5 globular BSA molecules can pack closely without repelling each other, resulting in high surface mass. When the system is returned to BSA at pH3 the thickness, mass and RI (density) return to approaching their original values indicating that the protein did not denature on the surface. Proteins retaining their globular framework tend to adsorb reversibly with respect to pH cycling whilst denatured proteins show irreversible adsorption.

Theses results demonstrate how the adsorption behaviour of different folded states of BSA can be distinguished using DPI measurements.

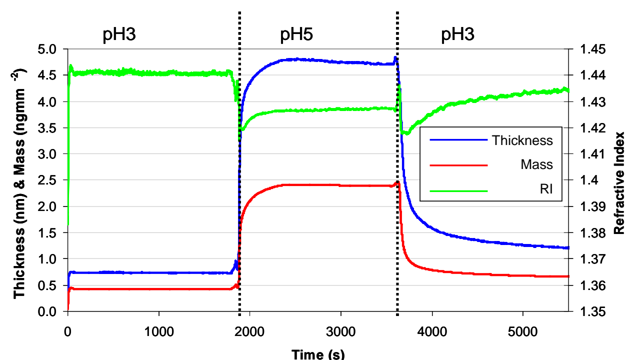

Figure 2: Reversible Conformational Change and Adsorption Behaviour Displayed by BSA during pH Cycling

Table 1 shows good correlation between DPI mass data at equilibrium and analogous neutron reflection data [1].

	[BSA] ($mgml^{-1}$)	Mass Adsorbed at pH3 ($ngmm^{-2}$)	Mass Adsorbed at pH5 ($ngmm^{-2}$)	Mass Adsorbed at pH7 ($ngmm^{-2}$)
Neutron Reflection	0.15	0.50	2.50	0.50
DPI	0.10	0.48	2.11	0.42

Table 1: Comparison of Adsorption of BSA at Equilibrium at Different pH Values Using Neutron Reflection and DPI

Looking at the dynamics of adsorption process there was an initial rapid adsorption of BSA at pH3 but a more gradual adsorption at pH7 implying different adsorption mechanisms [2]. The adsorption rate at pH5 was comparable to that at pH3.

Analysing the DPI data on the surface adsorbed structures, in combination with the measured adsorption rates and the known bulk solution structures, makes it possible to gain an understanding of the mechanisms involved in surface adsorption of BSA. At pH3 it seems that the adsorption process for the unfolded protein is predominantly driven by electrostatic interactions between the positively charged protein and the negatively charged ***Ana*Chip™** surface and occurs rapidly. The DPI thickness and density data imply that the protein orientates prone on the surface. At pH5, close to the IEP for BSA, adsorption is still rapid but slower than at pH3. Having no net charge at pH5 the globular molecules can pack closely together without repelling each other, resulting in high surface mass. At pH7 the molecules are negatively charged and the rate at which they interact with the ***Ana*Chip™** is reduced as electrostatic repulsion must be overcome before adsorption can proceed. Again they adopt a prone attitude on the surface.

Conclusions and Benefits

Many biological processes involve proteins changing structure (shape) as they function. ***Ana*Light®** gives the researcher a unique combination of high resolution data in real time on immobilised protein dimensions, density, surface coverage and orientation in a bench top technique. This application note clearly demonstrates that ***Ana*Light®** DPI systems offer powerful next-generation technology with the sensitivity and dynamic range needed to study pH dependent adsorption behaviour of proteins.

Ana*Light®** instruments have been successfully used to study a range of biomolecular, polymer and surfactant systems. With the capability of providing real-time data at a resolution commensurate with techniques such as neutron reflection and X-ray crystallography, yet remaining a benchtop technique, DPI is proving to be an invaluable tool for studying the intimate link between molecular ***structure and ***function***.

***Ana*Light®** is an important enabling tool for nanotechnologists and biophysicists, giving them the ability to:

- Measure the rate and affinity of adsorption onto a surface
- Quantify the structural and functional changes that result from pH effects on proteins at interfaces
- Determine reversibility (or otherwise) of conformational changes
- Obtain informative data from a convenient, laboratory-based technique

Farfield gratefully acknowledges that these experiments were carried out in collaboration with Professor Jian Lu, Biological Physics Group, School of Physics & Astronomy, University of Manchester, UK.

For further applications information contact: applications@farfield-group.com or Telephone the applications team on +44 (0) 870 950 9717

(1) Su *et al.*, ***J. Phys Chem***, B **102** (1998) 8100
(2) Freeman *et al.*, ***J. Phys.: Condens. Matter*** **16** (2004) S2493