APPLICATION NOTE

Assessment of protein stability and functionality by Flow-Induced Dispersion Analysis

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Introduction

Protein-based pharmaceuticals represents a rapidly growing class of drug compounds. However, development of protein-based drugs is associated with significant challenges as these complex molecules are structurally labile, and the drug molecule, vehicle or degradation products may cause immunogenic responses, thereby leading to loss of therapeutic effect, toxicity or even anaphylaxis. Current methodologies cannot address these risks, as they typically are unable to probe stability under native conditions and require large amounts of sample. In this work, it is shown that Flow-Induced Dispersion Analysis (FIDA) can be used for measuring the unfolding of Human Serum Albumin (HSA) and loss of binding affinity to Fluorescein under native conditions with minimal sample consumption.

FIDA is a new capillary-based technology for measuring binding affinity and assessing protein stability in-solution under native conditions. FIDA utilizes Taylor dispersion for accurate size determinations of analytes in a pressure driven flow system. The change in apparent size forms the basis for an accurate measure of binding affinity and protein stability.

Materials & Methods

FIDAlyzer instrument with 266 nm and 480 nm LIF and LED fluorescence detection for unfolding and binding experiments respectively (FIDA-Tech ApS). FIDA standard capillary (i.d.: 75 µm, L_T: 100 cm, L_{eff}: 90 cm). 67 mM phosphate buffer pH 7.4 was used as working buffer. HSA (0.5 mg/mL) as indicator in 0-7 M Urea for unfolding experiment. Fluorescein as indicator (10 nM), HSA as analyte (0-500 µM) in 0-4 M Urea solutions for binding experiments. Sample analysis was performed by filling the capillary with analyte, followed by injection of 39 nL indicator, which was mobilized towards the detector with analyte at 400 mbar.

Results

The size (hydrodynamic radius) of HSA was plotted as function of Urea concentration in Figure 1. It was observed that an increase in Urea concentration led to unfolding of HSA, observed as increase in size from 3.5 nm to 6.2 nm.

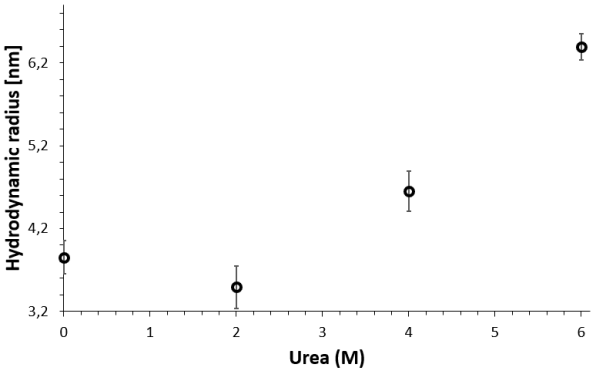

Figure 1: Unfolding curve for HSA in 0-7 M Urea.

Binding curves for the interaction between Fluorescein and HSA in 0-4 M Urea was estabilished (Figure 2). The binding affinity for the complex declines with increasing Urea concentration and thus associated with HSA unfolding and loss of binding functionality.

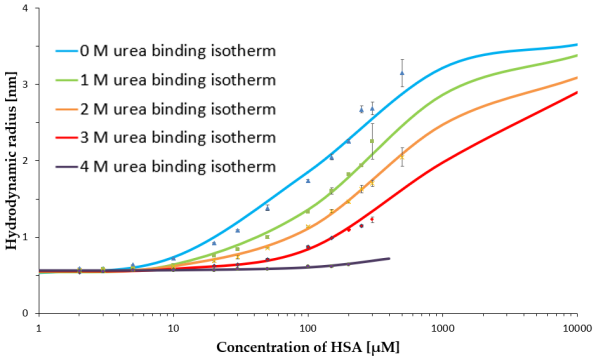

Figure 2: Binding curves for the interaction between Fluorescein and HSA in 0-4 M Urea.

Conclusion

The FIDA methodology is applicable for probing in-solution protein stability by measuring unfolding activities and loss of binding affinity with minimal sample consumption.

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