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

Key Fidabio benefits

- Rapid, HTP and efficient characterization of chemically induced unfolding of human serum albumin
- Assessment of local and global structural changes in protein unfolding event
- Data in good agreement with SAXS
- Simultaneous assessment of in solution binding affinity, protein stability and absolute size
- Native conditions and low amount of sample volume
- Built in quality control

Link to Tech-notes/App-notes

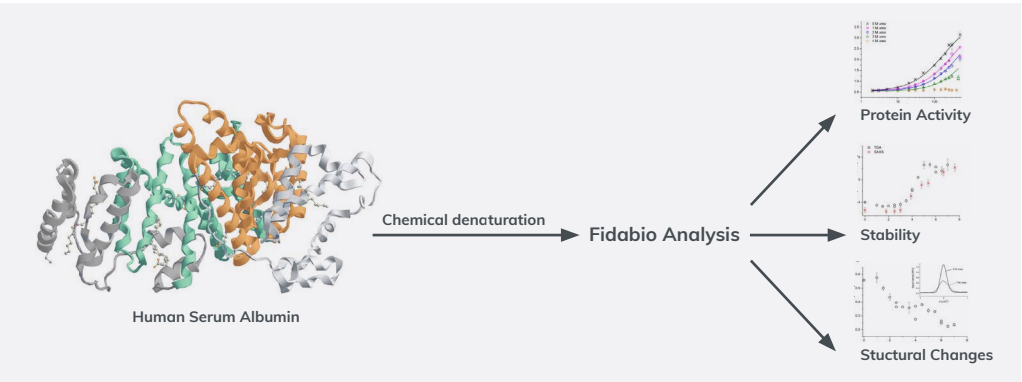
Version 01

Nadia Mirza, Sr. R&D application Scientist, Fida Biosystems

Introduction

The present application note is based on a peer-reviewed paper in 2019 (1). Protein-based pharmaceuticals represent a rapidly growing class of drug compounds. However, the development of proteinbased 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.



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Materials & Methods

FIDA 1 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, LT: 100 cm, Leff: 90 cm). 67 mM phosphate buffer pH 7.4 was used as the working buffer. HSA (0.5 mg/mL) as indicator in 0-7 M Urea for unfolding experiment.

Fluorescein as the 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 the analyte, followed by an injection of 39 nL indicator, which was mobilized towards the detector with analyte at 400 mbar.

Results

Urea-induced unfolding of HSA.

As FIDA technology provides an absolute measurement of hydrodynamic radius, it was used as a measure to address global changes upon HSA unfolding. Intrinsic fluorescence from HSA and its binding to a low molecular weight ligand - fluorescein - were probed to address local structural changes and functionality of the protein in varying urea concentrations.

The changes in the size (hydrodynamic radius) of HSA was plotted as a function of increasing urea concentration (0-7 M) at 25°C as shown in Figure 1A. An increase in Urea concentration around 4.0 M led to the unfolding of HSA, observed as an increase in the size of HSA from 3.5 nm to 6.2 nm. The results correlated well with a similar study using small-angle X-ray scattering (SAXS).

The peak areas of FIDA taylograms were exploited for simultaneously probing the intrinsic fluorescence intensity of HSA at increasing urea concentration. It shows that the intrinsic fluorescence of HSA was affected by urea at 1.5 M indicating local structural changes prior to the overall unfolding (Figure 1B), which is a unique capability of the FIDA method to measure both local and global structural changes in a single measurement.

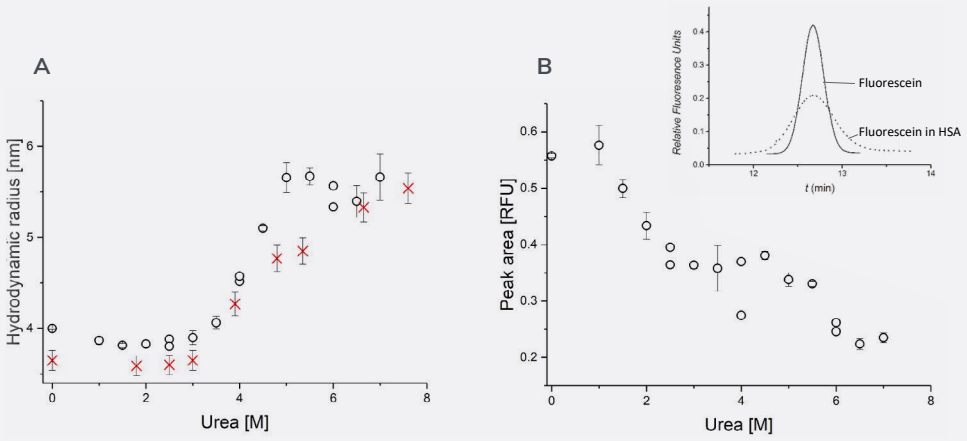

Figure 1: A, Unfolding curve for HSA in 0-7 M urea. *The hydrodynamic radius of HSA as a function of urea concentration determined by FIDA at 25°C and compared with SAXS.* **B**, *Intrinsic fluorescence area of 15 µM HSA as a function of urea concentration*

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Loss of HSA binding to Fluorescein. Binding curves for the interaction between Fluorescein and HSA in 0-4 M Urea were established (Figure 3) and the Kd values were obtained as shown in Table 1. The binding affinity for the complex declines with increasing urea concentration and thus associated with HSA unfolding and loss of functionality.

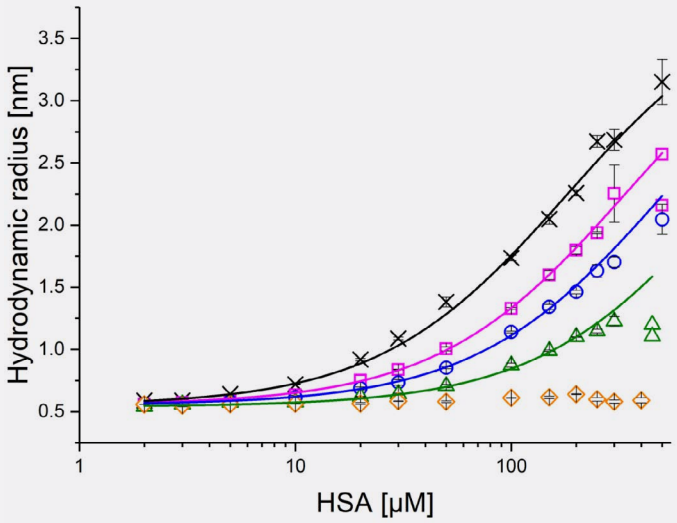

Figure 2: binding curves for the interaction between HSA and Fluorescein in 0-4 M urea

Table 1. Dissociation Constants (K_d) Obtained for the Binding of HSA to Fluorescein at Varying Urea Concentrations at pH 7.4 and 25 ^{0}C

Urea (M)	Kd uM	R^2 for binding isotherm fit
0	25,1	0,99
1	47,1	0,99
2	71,1	0,99
3	139	0,99

Conclusion

A combination of different techniques is required for a complete understanding of protein stability and function. The FIDA methodology is a multitiered approach capable of a detailed characterization of the denaturation and unfolding process as depicted by HSA unfolding induced by urea.

With minimal sample consumption and using one instrument, FIDA allows in-depth assessment of protein activity combined with local and global structural changes upon protein unfolding by measuring the overall hydrodynamic radius of the protein.

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References

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