Effects of Protein Dynamics on Aggregation Revealed by Hydrogen-Deuterium (H/D) Exchange Raman Spectroscopy Coupled with Dynamic Light Scattering

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**Purpose**

The aggregation propensity of therapeutic proteins has been studied extensively. However, most of these studies have focused primarily on the colloidal stability effects. Till date, investigations into the effects of protein dynamics has been very limited. However, in order to develop formulation design rules for optimizing protein stability, insights need to be generated on the effects of both the colloidal stability and protein dynamics aspects through obtaining protein size, structure/dynamics information on the same sample at the same time. Here, we provide an initial evaluation of above aspects via combined H/D exchange Raman spectroscopy with dynamic light scattering on a single platform.

**Methods**

Bovine Serum Albumin was purchased from Sigma. A pharmaceutical grade monoclonal antibody was used in its original formulation. For all the hydrogen exchange experiments, samples were concentrated in Amicon centrifuge filter to 300 mg/mL, then diluted to 30 mg/mL in 90% deuterium buffer. DLS-Raman experiments were performed on a Zetasizer Helix (Malvern Instruments). Raman scattering was excited by a 785 nm laser, while DLS was collected at 173° backscattering. Corresponding buffer were acquired under identical conditions.

**Results**

At 58 °C, BSA showed completely different spectra changes and aggregation pathway at pH 6.2 and 7.4. The overall spectra features showed more noticeable change at pH 7.4 than pH 6.2, for Amide I, III, and even disulfide bond region. A clear α-helix to β-sheet transition was observed at pH 7.4, but not at pH 6.2. Furthermore, the intensity change at low frequency was well correlated with the aggregation pathways. As for the mAb, although it aggregated, the spectra in aqueous buffer only showed minimum change. Significant peak shift in Amide I, III and intensity increase C-C stretch appeared in H/D experiments, indicating the backbone proton was exchanged. The disulfide bond region configuration also changed with the intensity increase at 528/542 cm⁻¹, indicating the observed spectra change could be a combination of H/D exchange and protein structure perturbation.

**Conclusion**

For the first time, protein/mAb aggregation, structure and even dynamics were evaluated in real time for the same sample at same time. Interesting results were observed for H/D exchange and corresponding aggregation/structure change.