The Effect of Fatty Acid Binding in the Acid Isomerizations of Albumin Investigated with a Continuous Acidification Method

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The most abundant plasma protein, Human Serum Albumin (HSA), is known to undergo conformational transitions in acidic environment. [1] To avoid artefacts due to multiple sample preparation and correlate global and local structural changes, we developed a continuous acidification method and simultaneously monitored the protein changes by both small-angle scattering (SAXS) and fluorescence (Figure 1), using a dedicated instrumental platform [2]. The time-dependent acidification, based on the hydrolysis of glucono-δ-lactone from pH 7 to pH 2.5, highlighted albumin's multi-step unfolding involving the putative F form (pH 4) and an extended and flexible conformation (pH < 3.5). [3]

In particular, the simultaneous fluorescence and SAXS characterization showed that the characteristic blue shift of the emission spectrum assigned to the native to F-form transition marked out the very first conformational rearrangements occurring with decreasing pH. The scattering profile of the F form was extracted by component analysis and modelled by low-resolution methods, suggesting the rearrangement of the three albumin domains in a more elongated conformation, with a partial unfolding of one of the outer domains at this intermediate stage (Figure 2).

The extent of binding of Fatty Acids (FA), which are albumin's physiological ligands, has been thought to have an impact on the conformational equilibrium between the native and acid forms and to be a possible explanation for the observation of more than one band in early electrophoretic migration experiments at pH 4. [4]

We therefore compared the acid-induced unfolding processes of commercial FA-free HSA, commercial "fatted" HSA and FA-HSA complexes, prepared at FA:HSA molar ratios between 1 and 6 by simple mixing and equilibration. The results showed that the presence of FA interacting with albumin affects the conformational response of the protein to acidification, and slightly shifts the loss of the native shape from pH 4.2 to pH 3.6 (Figure 3). [5] This effect increased with the FA:HSA molar ratio so that with three molar equivalents a saturation was reached, in agreement with the number of high-affinity binding sites reported for the FA. These findings confirm that a non-uniform level of ligand binding in an albumin sample can be an explanation for the early-observed conformational heterogeneity at pH 4. Our approach also showed how to realize a continuous, homogeneous and tunable acidification with simultaneous characterization applicable to study processes triggered by a pH decrease.

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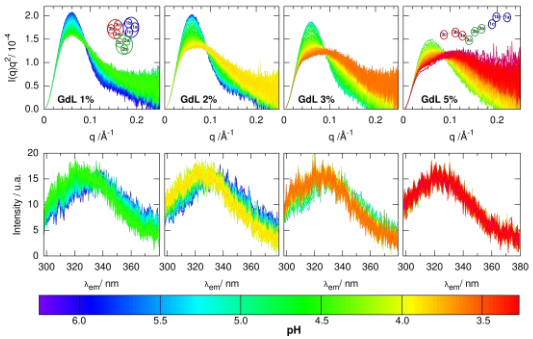

Figure 1. SAXS data (in the form of Kratky plot) and fluorescence emission spectra collected simultaneously for albumin samples acidified with different concentrations of glucono-δ-lactone.

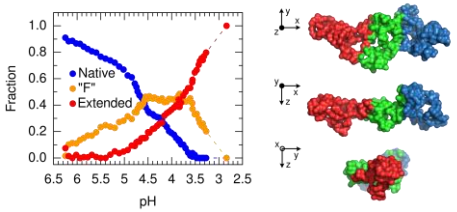

Figure 2. The SAXS data set of Albumin's unfolding can be described in terms of three conformational components whose fraction changes as a function of pH. A model for the intermediate "F" form could be obtained from SAXS data fitting.

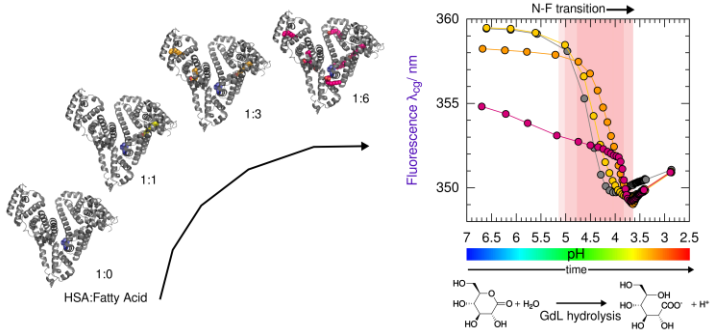

Figure 3. The transition from native to "F" state of albumin highlighted by the fluorescence blue shift occurs at slightly lower pH values in the presence of fatty acids.