Progressive Denaturation of Globular Proteins in Urea

Application Note

BioPharma

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Introduction

To be biologically active, proteins must adopt a specific three-dimensional, tertiary structure through non-covalent intramolecular interactions between amino acid groups in the primary structure. In this folded state, globular proteins are known to be compact and highly stable, requiring extremely harsh denaturing conditions to force them to unfold into a random conformation.

Protein denaturation involves alteration in the nature of the folded structure that results in changes to the biological, chemical and physical properties of the protein, manifestations of the conformational changes taking place in the polypeptide chain. The denaturation process can be achieved by increasing temperature, changing pH, the addition of denaturants (such as urea, guanidine hydrochloride and ß-mercaptoethanol), high pressure or the action of detergents (sodium dodecyl sulphate). In chemical denaturation, a chemical that is capable of forming stronger or equivalent bonds with the functional groups that maintain the conformation, is introduced. This disrupts the bonds holding the protein together. Urea and guanidine hydrochloride are strong denaturants, disrupting hydrogen bonding and thereby causing many proteins to adopt a highly unfolded and less compact conformation in solution. Some proteins aggregate in urea or guanidine chloride, usually due to the formation of disulfide bonds between sulfydryl groups made accessible by the unfolding of polypeptide chains.

Size exclusion chromatography (SEC) is an excellent technique for the investigating the effect of a denaturant on a protein, allowing analysis of aggregation and protein unfolding. This application note describes the analysis of a series of globular proteins using an Agilent ProSEC 300S column under denaturing and non-denaturing conditions to identify unfolded and/or aggregate species.

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Methods and Materials

Conditions

Column:	Agilent ProSEC 300S, 7.5 × 300 mm (p/n PL1147-6501)
Eluent:	Containing urea as specified 0.3 M NaCl, pH 8.0
Flow Rate:	1.0 mL/min
Injection Volume:	100 µL
Temperature:	5 °C
Detection:	UV at 310 nm + Agilent dual angle LSD

Results and Discussion

Figures 1-4 illustrate chromatograms of the progressive denaturation of a range of globular proteins in the presence and absence of urea. For each protein, two denaturing conditions were investigated: urea at a concentration of 2 M introduced for two hours and urea at a concentration of 8 M introduced overnight.

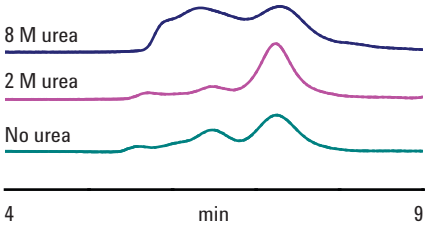

Figure 1. Overlay of UV for a sample of γ-globulins in differing urea conditions, illustrating an increase in aggregate/unfolded species formation with an increase in incubation time of protein with urea

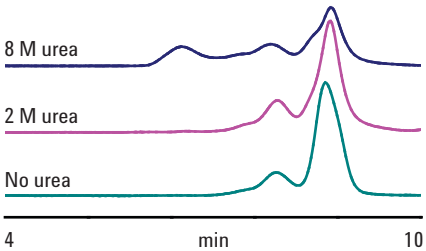

Figure 2. Overlay of UV for a sample of bovine serum albumen (BSA) in differing urea conditions, illustrating a decrease in monomer species and an increase in higher molecular weight species following incubation with urea

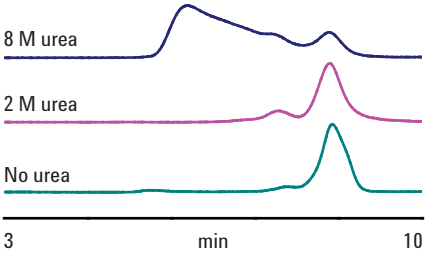

Figure 3. Overlay of UV for a sample of ovalbumin, illustrating a decrease in monomer species and an increase in aggregate species following incubation with urea

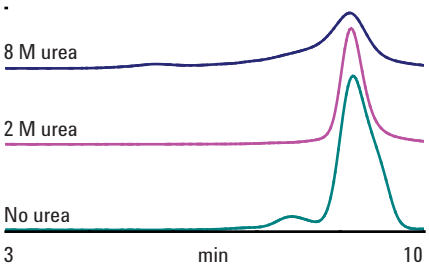

Figure 4. Overlay of UV for a sample of myoglobin, illustrating a decrease in monomer species and an increase in polymer species following incubation with urea

Each of the protein samples demonstrated that addition of denaturant resulted in a reduction in the amount of monomeric species in the sample, with concurrent increase in the quantity of higher molecular weight species, often in the form of a broad peak indicating the presence of aggregates. This indicates that in the case of the above globular proteins, denaturants such as urea instigated protein unfolding, producing higher molecular weight species due to the proteins adopting less compact structures, with aggregation through disulfide bond formation.

Conclusion

Analysis of globulins in denaturing and non-denaturing conditions using an Agilent ProSEC 300S column allowed the separation of proteins on the basis of their size in solution. Measuring the UV intensity identified multiple species in each of the protein samples and allowed the comparison of mixtures with and without denaturant.

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