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Stability Studies on Human Serum Transferrin

Formulation is a large challenge in development of biopharmaceuticals due the lack of fundamental understanding of protein behavior beyond the Hoffmeister series first presented in 1888. The PIPPI project is focused on protein formulation – investigating a database of proteins with diverse structural properties.

The current work focused on stability-structure studies of human serum transferrin using Nano-Differential Scanning Fluorimetry (NanoDSF), Isothermal Chemical Denaturation (ICD), light scattering techniques, and Small Angle X-ray Scattering (SAXS).

Transferrins (TFs) belong to a family of iron-binding proteins that play a crucial role in regulation of the iron levels. The primary role of TF is to transport the iron and supply the growing cells. The binding and release of iron is dependent of several factors, such as pH, ionic strength, and chelators that have an influence on protein stability.

NanoDSF allows for monitoring protein stability with increasing temperature and provides information about the melting temperature (T_m). ICD allows for monitoring chemical unfolding of the protein with increasing of denaturation agent concentration. From ICD data several parameters might be calculated, such as Gibbs energy of unfolding (ΔG), $C_{1/2}$ and m-values.

The overall stability and behavior of transferrin was investigated at varying pH and ionic strength using nanoDSF and ICD. The most interesting conditions were selected and the structure was analyzed by SAXS. The results were further explored using Static Light Scattering (SLS) and SEC-MALS (Size Exclusion Chromatography with Multi-Angle Static Light Scattering detector).

The results from the nanoDSF and the ICD measurements show that the conditions for thermal and chemical stability may be different. Furthermore, we demonstrate that X-ray and light scattering complement each other in giving the full picture of the solution structure of transferrin.

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