Long Range Interactions of Apoferriten in Solutions Revealed by SAXS

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SAXS for protein solutions of Apoferriten were measured with the SAXS instrument at the NSRRC. The instrument was described in a previous report: "An instrument for time resolved and anomalous simultaneous small and wide angle X-ray scattering (SWAXS) at the NSRRC", *Journal of Applied Crystallography*, 39, 871-877 (2006). The sample to detector distance used was 2270 mm, and the 10 keV beam size used was of 0.5 mm dia.. The preliminary results are summarized below.

(1) Temperature dependence

Temperature-dependent SAXS profiles measured for the protein sample solutions of 5% and 10% Apoferriten in water.

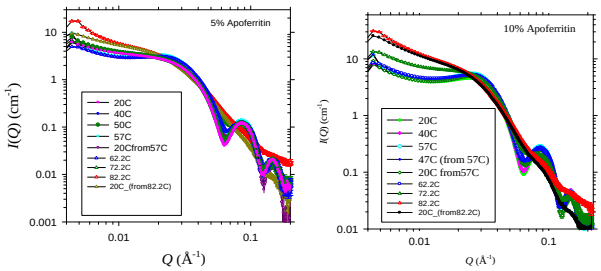

Figure 1. Temperature dependent SAXS profiles for the protein solutions, 5% and 10%, without salt.

(2) Salt dependence

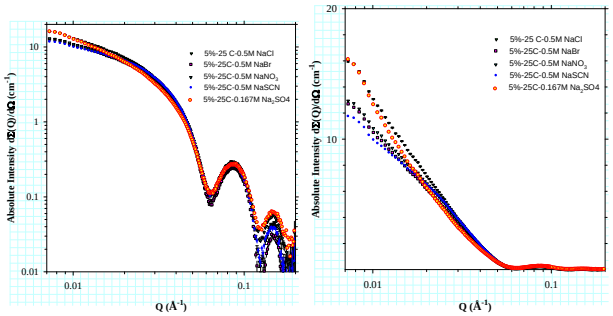

Figure 2. Two presentations of the salt dependent SAXS data for the protein solutions. The first and second structural peaks of the protein molecule are clearly observed in the intermediate and high Q range, whereas the protein cluster peak at low Q shows the strong dependence on the salt used.

(3) Concentration dependence

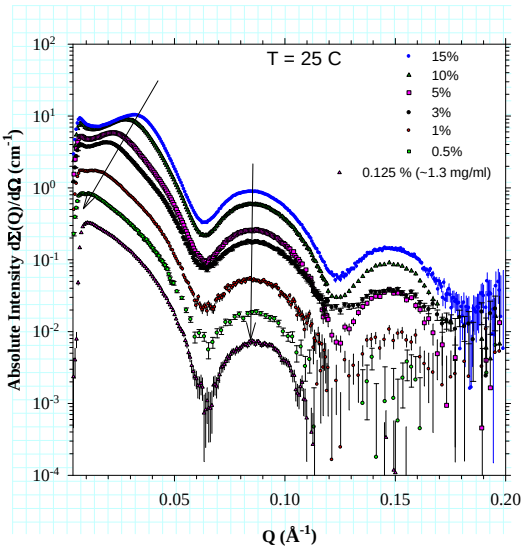

Figure 3. Concentration dependence of the SAXS data indicate that the structural peaks of the individual Apoferritin protein molecule are independent of protein concentration. In contrast, the protein cluster peak shifts to a lower Q value due to the narrower cluster spacing as the protein (cluster) concentration increases.

(4) Conclusions

These very interesting results are in agreement with Prof S. H. Chen's theory on the presence of a long range attractive interaction between proteins. The protein form factor are quite stable for temperatures with all the salts present in the temperature range measured (20 - 60 C). But the clustering or interactions seem to change with temperature (when T>60 °C), and such changes were not reversible, at least not for a short time of more than 0.5 hour that we have waited.

Above 60 °C, the form factor starts to change, leading to a kind of irreversible denaturation. As the temperature is increased to 80 °C, all the proteins with salts tend to form a fractal like structure, and the form factor nearly lost completely signaling a complete denaturation (see F. Mallamace et al. **JCP** 127, 045104, 2007) The transition seems to be an irreversible process, at least in the short time interval of the measurements. The best cluster ordering occurs at ~57 °C (without salt), right before the changing of the form factor or protein unfolding.