ESRF

Experiment title:

Pressure induced unfolding in proteins

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Report:

High pressure effects on proteins

In this report we focus on results, which have been derived from high pressure SAXS experiments on ribonuclease A. Similar experiments have been performed on Lipoxygenase and Human interferon γ and information on gyration radii of these proteins could be derived as well. However, presently limitations in the lower end of the q-regime did not allow us to perform a more detailed investigation of pressure influence on dimension and shape in case of this larger proteins.

Using high pressure FTIR spectroscopy, a conformational transition of RNAse A was detected araound 7kbar by investigating absorption from tyrosine side chains and amide II. This has been interpreted in terms of an unifolding process. In addition, a weak "pretransition" araound 4kbar was detected by observing amide I and II absorption, which suggested to us that a transition frorn the compact to unflolded state proceeds -unlike temperature unfolding- via a swolen intermediate state, where the dimensions of the protein increase, but the secondary structure remains intact.

Using the high pressure cell at ESRF, we performed SAXS experiments in order to gain insight in pressure-induced changes of dimension and shape of RNAse A. Data have been collected in a q-range from 0.03 to 0.25 with pressure steps of 0,6kbar and corrected for background signal, arising from the high pressure cell and buffer solution.

In order to get information about the protein dimensions, we calculated gyration radii from Guinierplots. The R_G values, derived at pressures below 7kbar are in the range of 15 to 17Å, close to the values reported in literature for RNAse native. A slight increase to about 20Å has been detected during pressure increase above 7kbar. However, Guinieranalysis only makes use of the low angle part of the scattering diagram. From fourier transforms of the full scattering pattern we calculated the distance distribution functions for various pressures, see enclosed figure. The distance distributions revealed a consistent shift of the maximum value towards lower values during pressure increase. Furthermore, a small shoulder evolves continuously during pressure increase which might be taken as indication for an unfolding of the protein. As judged from Kratkyplots of the intensity data, the shape of the proteins remains rather compact at high pressures, even if there is some consistent depression of the maximum in these plots, which might indicate evolution to a more irregular shape.

All the results indicate that small, continuous changes can be observed in proteindimension and shape. These properties seem to proceed in a more continuous way, whereas our high pressure FTIR experiments indicated distinct confirmational transitions affecting secondary structural elements. Thus, it seems necessary to extend the investigations to the high-q range where scattering contributions of the secondary structure may appear.