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Report:

The purpose of the small angle x-ray scattering (SAXS) experiment SC-2403 was to investigate the structure and the unfolding properties of various mutants of the model protein staphylococcal nuclease (SNase) in aqueous solution by application of hydrostatic pressure and in different chaotropic and kosmotropic cosolvents. Pressure dependent were carried out to explore the stability of the proteins in the full p,T-phase space. Furthermore, the stability was tuned by single residue substituation. A home-built hydrostatic high-pressure sample cell was used throughout our studies [1]. With this we were able to perform temperature and pressure dependent measurements up to pressure of about 4000 bar.

Measurements were performed on the mutants V66R, V66A and V66Y of Δ+PHS, the hyperstable form of SNase, at pH = 5.4. Throughout these experiments, the protein concentrations were 1% wt. To avoid pressure-induced changes of the pH-value, 50 mM bis-Tris buffer was used.
To cover a wider q-range, two different sample-to-detector distances (0.91 m and 3.04 m) were choosen.
Data of the native proteins in pure buffer solution were acquired at a temperature of 35°C in a q-range from 0.3 nm^{-1} to 3.5 nm^{-1} with high quality. In this range it is possible to obtain the form factors of the mutants up to the first maximum and the radii of gyration R_g by a Guinier plot and by means of indirecct Fourier transform. Under these conditions, at least in two cases (V66A, V66Y), the mutants being observed are in their native state.

To unfold the proteins, high pressures up to 4000 bar were applied, since it is known that the less stable wild type protein unfolds at pressures of about 2500 bar [2,3]. To further determine how different cosolvents influence the denaturation process, measurements were made with solutions of 1.5 M and 2.5 M urea and 0.5 M and 2.5 M glycerol added to the pure buffer solution.

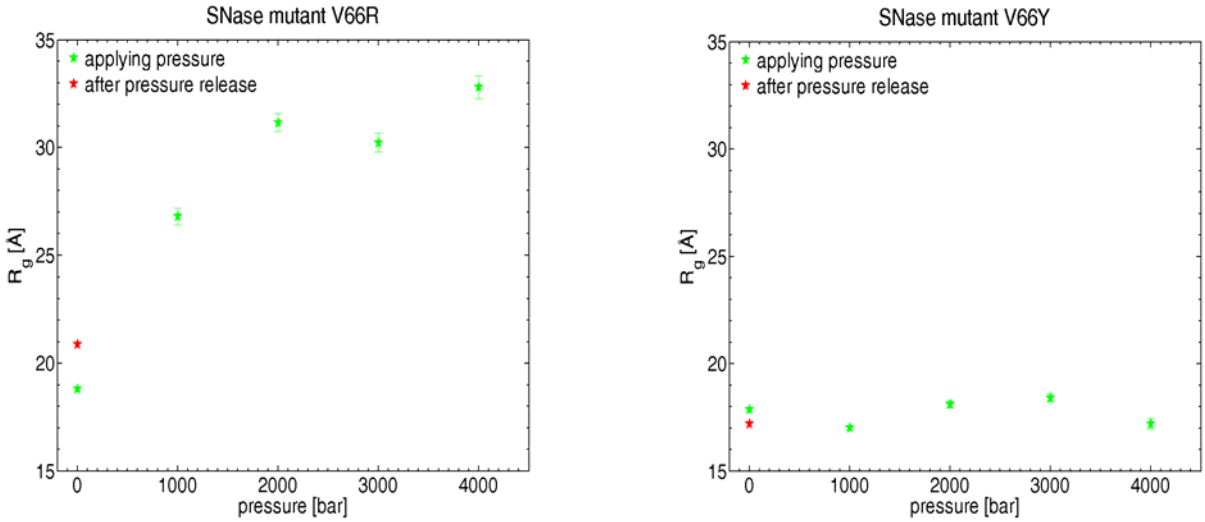

Figure 1: Radii of gyration R_g as a function of pressure for two mutants of SNase in 50 mM bis-Tris buffer at T = 25°C. Obviously, the V66Y mutant is more stable against pressure denaturation than the V66R mutant. Data were obtained at a sample-to-detector-distance of 3.04 m at 12.5 keV.

In Fig. 1, the evolution of R_g as a function of pressure for two mutants in pure buffer solution is shown exemplarily. As can be seen, there are differences concerning the stability.
The mutant V66R is denatured by a pressure of about 1 kbar, whereas R_g V66Y does not change at all. In both cases shown, releasing the pressure yields a radius of gyration which is almost the same at the beginning of the experiment. This indicates that the refolding process of the proteins is largely reversible. Measurements on V66A show a similar behaviour to V66Y.

Applying high pressure to a solution of V66A under denaturing condions using urea leads to a drastic increase of R_g at 4000 bar. Under pure buffer conditions, no such unfolding is observed as mentioned above. Thus, urea significantly destabilizes the mutant towards unfolding also under high pressure conditions. When enhancing the urea concentration a small increase of R_g is observed before the final unfolding state is reached.

Addition of glycerol, a stabilizing cosolvent, does not cause any significant changes compared to the pure buffer solution due to stabilization of the protein. Hence, also no effect is obsevered when increasing the glycerol concentration.

Apart from high pressure measurements, the mutants were also studied at 64.0°C, a temperature well above the unfolding temperature of the wild-type protein. The data obtained indicate that V66R is denatured under these conditions. Also, aggregation seems to have taken place. The same holds true for V66A, however, with a less dramatic increase of the radius of gyration.
In contrast, the SNase mutant V66Y seems to be stable against heat denaturation up to these temperatures.

Summarising, we investigated the stability of three SNase mutants towards pressure unfolding in buffer solutions and in solution with different cosolvents. We further inverstigated the effect of high temperatures.
Our data suggest that the mutant V66R is the least stable variant studied. V66A does not unfold in pure buffer in the pressure range investigated. V66Y does not denaturate under the thermodynamic conditions studied.

Further data analysis and a publication are in progress.

References

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